ACADEMY OF SCIENCES OF THE U. S. S. R.

D. N. NASONOV

Local Reaction of Protoplasm and Gradual Excitation

TRANSLATED FROM RUSSIAN

Published for the
National Science Foundation, Washington D.C.
and the Department of Health, Education and Welfare
by the Israel Program for Scientific Translations

Prof. Traskin: Dec 11, 1963

It gives me great pleasure to present you with this English translation of Prof. Nasonov's book.

AKADEMIYA NAUK SSSR. INSTITUT TSITOLOGII
Academy of Sciences of the U.S.S.R. Institute of Cytology

D. N. NASONOV

Local Reaction of Protoplasm and Gradual Excitation

(Mestnaya reaktsiya protoplazmy i rasprostranyayushcheesya vozbuzhdenie)

1442

Izdatel'stvo Akademii Nauk SSSR
Moskva-Leningrad
1959

Translated from Russian

Published for the National Science Foundation, Washington D.C., by the
Israel Program for Scientific Translations,
Jerusalem 1962

OTS 61-31011

Published for
THE NATIONAL SCIENCE FOUNDATION, WASHINGTON, D.C.
and
THE DEPARTMENT OF HEALTH, EDUCATION AND WELFARE, U.S.A.
by the
ISRAEL PROGRAM FOR SCIENTIFIC TRANSLATIONS
Copyright©1962
Israel Program for Scientific Translations, Ltd.

Translated by Y.S. Halpern, Ph.D., M.Sc.

Printed in Jerusalem by S. Monson

PST Cat. No. 546

Price $4.25

Available from the Office of Technical Services
U.S. Department of Commerce, Washington 25, D.C.

On the basis of a great number of foreign and Soviet studies and monographs, an analysis is given of the fundamental patterns of cell activity.

The author develops the protein theory of cell injuries and excitation, analyzes the membrane theory and outlines the sorption theory of cell permeability and bioelectric phenomena.

Particular attention is given to the theory of gradual excitation, in which the causes of and conditions for transformation of local bioelectric potentials into spreading potentials are considered. The work also deals with the problem of excitability of nerve and muscle fibers.

The book is intended for biologists and medical research workers (cytologists, physiologists, biochemists, pathophysiologists, toxicologists, pharmacologists, etc.).

TABLE OF CONTENTS

Dmitri Nikolaevich Nasonov 1
Foreword 4

PART I. LOCAL REACTION

Chapter 1. Reaction of Living Protoplasm to External Influences 5
Paranecrosis 5
Denaturation theory of excitation 9
Chapter 2. Quantitative Evaluation of the Degree of Alteration of Cells by the Action of Stimuli 12
Increase in sorption capacity of native proteins due to the influence of denaturating agents 12
Method for determining the extent of alteration of living protoplasm by the action of stimuli 20
Audible sounds as direct stimuli of cellular protoplasm 25
Reaction of protoplasm to high hydrostatic pressure 34
Chapter 3. Local Reaction and Stimulation 40
Excitation and damage 40
Excitation, damage and narcosis of skeletal muscles 44
Local excitation and contraction of connective tissue cells 62
Metabolism during local excitation 66
Certain features of paranecrosis during physiological stimulation of cells 75
Paranecrosis and stimulation of gland cells 86
Decrease in vital staining of protoplasm 92

PART II. EXCITATION AND PERMEABILITY

Chapter 1. The Membrane Theory of Cell Permeability and its Critical Appraisal 95
Cell permeability and the theory of excitation 95
Origin and development of the membrane theory of permeability 96
The hypothetic cell membrane and its suggested chemical composition and structure 97
The osmotic properties of the cell 100
Chapter 2. Basis of the Sorption Theory of Cell Permeability 111
Penetration of nonelectrolytes into the cells from the point of view of diffusion and distribution phenomena 111
Analysis of distribution curve. Studies by Troshin with nonelectrolytes 116
Distribution of aminoacids between the cells and the medium 126
Distribution of vital stains between the cells and the environment 129
Nonequilibrium of electrolyte composition of cells and environment 133
Permeability of erythrocytes to electrolytes 135
Permeability of muscle fibers to electrolytes 136
Permeability of nerve fibers to electrolytes 139
Electrolytes in the protoplasm of nonstimulated cells 140
State of electrolytes in protoplasm after excitation 148
The mechanism of distribution of mineral substances between the cell and environment 150
Chapter 3. Phase Properties of Protoplasm 158
Immiscibility of living protoplasm with surrounding water. Attempts to explain this phenomenon 158
Distribution of substances between coacervates and their equilibrium liquid 159
Chapter 4. Discussion and Conclusions 163

PART III. BIOELECTRIC POTENTIALS

Chapter 1. Phase Theory of Bioelectric Potentials 170
History of the problem 170
Salt potentials 171
Injury potentials and action potentials 176
Chapter 2. Arguments in Favor of the Phase Theory 179
Site of the potential spike occurring after injury to the protoplasm 179
Increase in the "lowered injury potential" on renewal of the cut 181
Different rates of decrease in injury potential in Ringer's solution and in the moist chamber 184
Increase in injury potential of muscles after cutting 187
Relationship between the method of cutting and the magnitude of injury potential 189
Injury potential exceeded by action potential 190
Chapter 3. Bioelectric Potentials and Cell Metabolism 195
Statement of the problem 195
Effect of inhibition of different cellular metabolic processes on bioelectric potentials 196
Relationship between bioelectric potentials and cholinergic activity of cells 197
Oxidation-reduction theories of bioelectric phenomena 199
Phase theory of bioelectric potentials and cellular metabolism 201

PART IV. SPREADING EXCITATION AND ITS CONNECTION WITH THE LOCAL REACTION

Chapter 1. History of the Problem 203
Chapter 2. The Theory of Gradual Spreading Excitation 207
The electric mechanism of transmission of excitation 207
Basic principles of theory of gradual excitation 210
Supramaximal potentials 215
Absolute and relative refractory phases in the spreading excitation 228
Absolute and relative refractivity on stationary stimulation or narcosis 231
Conduction of impulse with decrement in a narcotized nerve 237
Spontaneous rhythmic activity of conducting fibers 253
Conclusions 267

PART V. EXCITABILITY AND ITS MEASUREMENT

Chapter 1. Excitability Constants 269
Determination of excitability threshold 269
Relationship between threshold voltage, or intensity of stimulation current, and the time of its action 270
Physiological significance of constants a and b in the formula of Hoorweg-Weiss 272
Gradient factor (constant n) 275
Chapter 2. Measurement of Excitability 279
Method of measuring 279
Method of determination of excitability of human muscles 283
Comparison of excitability of various tissues 284
Chapter 3. Chronaxy 287
Does chronaxy determine the rapidity of excitation? 287
Examples of errors in chronaximetry 288
Certain disputable questions 294
What is chronaxy? 296
Chapter 4. Change in Excitability on Sectioning a Nerve 298
Historical aspects of the problem 298
Materials and methods 299
Experiments with frog nerve 299
Experiments with rat nerve 301

Chapter 5. Effect of Temperature on Excitability of Nerves and Muscles 306
Experiments with nerves of cold-blooded animals (frogs, tortoises) 306
Experiments with nerves of warm-blooded animals (rats, pigeons) 316
Experiments with skeletal muscles of frog and rat 322
Chapter 6. Conclusions 325

PART VI. CONDITIONS FOR THE APPEARANCE OF SPREADING EXCITATION IN A CONDUCTING FIBER

Chapter 1. Importance of the Amount of Electricity and Energy of Stimulating Current During the Appearance of Excitation 326
Theory of the physiological action of electric current 326
Effect of interelectrode distance on excitability threshold 331
Importance of voltage, intensity and energy of excitatory current for appearance of excitation in the nerve fiber 335
Experiments with isolated fibers 341
Discussion 343
Chapter 2. Electrotonus and Accomodation 346
Electrotonus of Pflügler 346
Verigo's concept of Pflügler's electrotonus 347
The theory of Verigo and so-called accomodation 350
Studies confirming Verigo's theory 353
Origin of spreading excitation 358

Conclusions 361

Closing Notes 365

Bibliography 367

Author Index 404

Subject Index 410

3

DMITRII NIKOLAEVICH NASONOV

(Short Biography)

Dmitrii Nikolaevich Nasonov was born in Warsaw on 10 July 1895, the son of Nikolai Viktorovich Nasonov, a zoology professor who later became an Academician. In 1912 he completed his secondary school studies in Petersburg and entered the Faculty of Physics and Mathematics at the Petersburg University, where in 1919 he took his degree in Natural Sciences. During his student years Nasonov, under the guidance of the well-known histologist A.S. Dogel', completed his first scientific work "Cytological Studies of Plant Cells"*, for which he was awarded a golden medal.

After completing his university studies, he worked as assistant and, from 1929, as lecturer in the Department of Histology. During this period he completed a number of original studies on the structure and function of the Golgi apparatus. He showed that the Golgi apparatus plays an important role in the secretory activity of the cell, and that certain substances which enter the cell from without, accumulate in the region of the reticular apparatus. The works of Nasonov on the Golgi apparatus were classics of their kind, and his name became widely known in international scientific circles.

In 1926 D.N. Nasonov was granted a Rockefeller scholarship which enabled him to visit Columbia University (New York) where he worked for one year in the laboratory of the well-known cytologist E.B. Wilson.

From the very beginning of his research activity Nasonov showed a deep interest in the study of the cell physiology. In the early 30's, he directed his investigations to reactions of living cells to external influences.

In 1932, at the invitation of A.A. Zavarzin, he became the director of the Cytological Laboratory of the Department of General and Comparative Morphology of the All-Union Institute of Experimental Medicine. Somewhat later, he was invited by A.A. Ukhtomskii to head the Laboratory of Cell Physiology in the Physiological Institute of the Leningrad University.

---

* [Tsitologicheskie issledovaniya nad rastitel'nymi kletkami]

In 1935, D.N. Nasonov was awarded the academic degree of Doctor of Biological Sciences, and the title of Professor.

This period of his life was scientifically very productive. The team headed by him successfully worked out important problems of cell physiology; they formulated the "protein theory" of cellular damage and excitation, indicating the leading role of protein in cell physiology. It was established that as a result of various influences on the cell, nonspecific or specific changes termed "paranecrosis" occur in the protoplasm. The basis of this reaction was a change in cell proteins, similar to the initial phases of protein denaturation. At the same time, a quantitative method was elaborated for the determination of the functional state of different tissues by the binding property of protoplasm for vital stains, a method which found wide use in biology and medicine. All these works were compiled in the monograph by D.N. Nasonov and V.Ya. Aleksandrov (Reaktsiya zhivogo veshchestva na vneshnie vozdeistviya -The Reaction of the Living Organism to External Influences, 1940) which was awarded a Stalin prize.

The study of cell reaction to external stimuli led to the revision of well-established concepts in general physiology, such as the membrane theory of permeability and the membrane theory of bioelectrical potentials. Nasonov and his team provided an experimental basis for a new approach to these phenomena, whereby the leading role was ascribed not to the semipermeable membranes, but to the cytoplasmic proteins (the phase theory of permeability and bioelectric potentials).

D.N. Nasonov combined intensive experimental work with wide teaching activity. In the early 30's, he established an original lecture course on cell physiology, which he read at the Leningrad University until the last year of his life, and which invariably drew the attention of students and specialists.

In July 1941, when the German armies approached Leningrad, Nasonov, then a known scientist, volunteered for the Red Army. For his participation in the battles of Leningrad, he was awarded the medal "For Combat Achievements". In the summer of 1942 Nasonov was wounded, and after recovery he was discharged.

In 1943-1944, D.N. Nasonov occupied the Chair of Histology at the Moscow University. In 1943 he was elected Member-Correspondent of the Academy of Sciences of the U.S.S.R. and in 1945 he was elected to active membership of the Academy of Sciences of the U.S.S.R.

Upon his return to Leningrad in 1944, D.N. Nasonov headed the Department of General and Comparative Physiology at the Leningrad University, and continued as director of the laboratory at the Institute of Experimental Medicine. From 1945, after the death of A.A. Zavarzin, he became director of the Section of General Morphology.

In the postwar period the scientific creativeness of Nasonov was especially intensive. He continued to study the substantial changes in protoplasm in response to various influences, since it had been shown that the normal activity of nervous, muscular, glandular, and other tissues is also accompanied by paranecrotic changes. However, it has been established that paranecrosis and parabiosis, as described by N.E. Vvedenskii, are the organic and functional aspects, respectively, of reactions of living creatures to external influences.

The vast material accumulated by the team headed by Nasonov contained convincing evidence of a quantitative relationship between the

magnitude of the response reaction of the cell and the strength of the stimulus acting on it. These facts were developed in the theory of gradual excitation, on which D.N. Nasonov worked during the last decade of his life.

5 This theory established the identical properties of local and spreading excitation. The basic forms of nervous activity and their development during changes in the functional state of conducting tissue are explained by this theory.

In addition to the theory of gradual excitation, Nasonov in his last years solved a number of other general physiological problems connected with the laws of electrical stimulation of tissue, and the effect of temperature on excitability, etc. His postwar works were summed up in a new monograph: Mestnaya reaktsiya protoplázmy i rasprostranyayushchesya vozbuzhdenie (The Local Reaction of Protoplasm and Spreading Excitation, 1957).

D.N. Nasonov was a great Soviet scientist, the founder of a new progressive trend in cytology and physiology, a talented teacher who trained a large team of specialists in cytophysiology. He was always a tireless worker. From his student years and until the last days of his life, he devoted daily a great deal of time and energy to experimental studies.

Dmitrii Nikolaevich was a militant scientist, who participated in numerous discussions in a spirit of high scientific principles and patriotism. He always defended the progressive materialistic trend in Soviet biology.

His scientific and teaching activities were always combined with extensive social and organizational work. From 1939 to 1950, he was a Deputy of the Vasil'evskii Island Regional Soviet of the Workers' Deputies. He was Dean of the Faculty of Biology at Leningrad University (1940 and 1941) and Director of the Institute of Experimental Medicine of the Academy of Medical Sciences of the U.S.S.R. (1948-1950).

In 1957, the Presidium of the Academy of Sciences of the U.S.S.R. entrusted him with the organization of a new Institute, the Institute of Cytology, modeled on the Laboratory of Cytology of the Zoological Institute of the Academy of Sciences of the U.S.S.R. in Leningrad, which was headed by Nasonov from 1955. He planned the structure of the Institute, chose a highly qualified staff, and planned its scientific projects. In March 1957, the Institute of Cytology began to function under his direction. Dmitrii Nikolaevich Nasonov suddenly passed away on 21 December 1957, at the height of his career.

A.S. Troshin.

9

FOREWORD

This book contains further developments on the ideas presented in the monograph "Reaction of the Living Organism to External Influences", (Nasonov and Aleksandrov, 1940). In this monograph, our conclusions were based mainly on microscopic observations made by our co-workers, by other investigators, and by us. These conclusions were that in the protoplasm of various cellular elements, a complex process of similar reversible changes occurs at the site of stimulation as a result of various external factors. This complex process was named "paranecrosis"

A detailed study of this nonspecific reaction led us to the conclusion that it is brought about by reversible changes in the protoplasmic proteins, similar in nature to denaturation of proteins in vitro.

At that time we tended to treat cellular paranecrotic changes mainly as reversible injuries, and we therefore gave the book the subtitle "The Denaturation Theory of Damage and Excitation". However, in one of the last chapters we expressed the opinion that the reversible protein reaction is most probably the result not only of reversible injury to the protoplasm, but also of its physiological excitation. We wrote: "At present, we cannot indicate convincingly enough the natural relationships between the appearance and conduction of nerve excitation, and appearance and irradiation of the primitive paranecrotic reaction of the cell to external influences. Nevertheless, a number of data forecast that further work in this direction will force us to realize the close association of these phenomena" (Nasonov and Aleksandrov, 1940, p. 204).

In the postwar years, the work of our team was mainly directed towards the confirmation of this assumption, and the present book is an attempt to sum up these investigations.

My heartfelt gratitude is hereby expressed to my dear comrades at work—V. Ya. Aleksandrov, A.D. Braun, N.V. Golovina, A.V. Zhirmunskii, I.E. Kamnev, M.B. Kiro, D.L. Rozental', S.N. Romanov, I.P. Suzdal'skaya, A.S. Troshin, V.P. Troshina. B.P. Ushakov, E.A. Shapiro, and others, whose work formed the basis of this book.

D. Nasonov

Leningrad, December 1956.

Part I

LOCAL REACTION

Chapter 1. Reaction of Living Protoplasm to External Influences

Paranecrosis

Any living cell, be it a unicellular organism or a part of a multicellular structure, is in constant interaction with the external environment.

If environmental factors (temperature, radiation, chemical composition, mechanical effect, barometric pressure, etc.) for any reason rapidly change their intensity, exceeding the limits of physiological norm for the given cell, then characteristic reversible changes occur in the cell protoplasm. These may be detected by different physiological or biochemical methods, or by observation of the living substrate under the microscope.

Such environmental changes to which the living organism reacts we shall arbitrarily call stimulants. The ability of the living substrate to react to the stimulants by a certain complex of changes we shall call excitability.

The reversible complex of changes observed at the site of action of the stimulant is sometimes given the general term "local change" or "local reaction". N.E. Vvedenskii, who studied these changes in detail, called them "local stable excitation", or "parabiosis". These terms often led to disputes and misunderstanding, and therefore their use requires justification. In this connection, at the beginning of this treatise, we shall use the less defined term "local reaction" and, subsequently, after giving the necessary factual data, we shall establish a basis for the term "local excitation"

In the present book, in order to avoid repetition, we shall state only the basic assumptions connected with this problem and we shall consider in detail only those studies carried out after our monograph was published.

The action of the following agents on living cells was studied and reviewed from data in the literature: increased temperature (Nasonov, 1932a; Braun and Ivanov, 1933; Aizenberg, 1934; Meshcherskaya, 1935); mechanical influences (Raevskaya, 1948); radiant energy (Aleksandrov, 1934); hydrostatic pressure, the cathode and anode of electric current (Kamnev, 1941, 1948a, 1948b, 1948c, 1949); substances acting at the
12 cell surface—narcotics (Makarov, 1934, 1935, 1936a, 1936b, 1938; Nasonov and Aleksandrov, 1937; Raevskaya and Troshin, 1937; Suzdal'skaya, 1948a)—acids (Nasonov, 1932a; Braun and Ivanov, 1933);—alkalis (Braun and Ivanov, 1933; Nasonov and Aleksandrov, 1934); excess and deficiency of neutral salts—hypertonicity and hypotonicity (Braun and Ivanov, 1933; Kamnev, 1934a, 1934b, 1936; Meshcherskaya, 1935; Nasonov and Aleksandrov, 1937; Raevskaya and Troshin, 1937); salts of heavy metals,

oxygen deficiency (Nasonov, 1930; Aleksandrov, 1932, Braun and Ivanov, 1933; Makarov, 1934; Meshcherskaya, 1935, 1939, Trifonova, 1935, and others*).

Recently we have observed and investigated the direct effect of sonic waves on living protoplasm. In the laboratory of Ginetsinskii a most interesting study has been performed on the effect of acclimatization of mice to oxygen deficiency, by vital staining of their muscles (Barbasheva and Ginetsinskii, 1956). This effect was studied in epithelial, nerve, muscle, connective tissue, and gonadal cells of vertebrate animals; in nerve and other cellular elements of worms, echinoderms, coelenterates, molluscs, crustaceans, insects, and other invertebrates, and also in representatives of protozoa and in certain cells of plant organisms.

First of all our attention is drawn to the fact that the most varied physical and chemical stimulants, sometimes utterly different in nature, cause the same complex of changes when they react singly on the cell. Therefore, the local response reaction of the protoplasm may be called a nonspecific reaction.

What are the changes appearing in the "stimulated" protoplasm?

It is apparent from many studies that one of the main effects is a decrease in the degree of dispersion of colloids present in the cytoplasm and nucleus. This fact is best detected in a dark field, where, in response to stimulation, all of the protoplasm firstly acquires a pale-blue glow, with subsequent appearance of bright white structures. On investigation with an ordinary microscope illuminated by transmitted light, the decrease in the degree of dispersion of protoplasmic colloid is manifested in the appearance of turbidity, and in the detection of visible structures in the cell. This is especially noticeable in the nuclei, which in normal cells (in the majority of cases), may be detected only with difficulty by the light contour surrounding them. The remaining part of the nucleus is more often than not structureless, and as many observers say, "optically empty". Following the action of any stimulant, structures which were not previously visible sometimes appear in it even earlier than in the protoplasm. The nuclear framework and chromatin particles become visible, and the nucleus acquires the appearance familiar from fixed preparations.

Another general feature characterizing "stimulated" protoplasm is the change in its viscosity, which may increase to a great extent under the influence of various agents. The change in viscosity is often biphasic: at the beginning, after the action of small doses of stimulant, viscosity may decrease, and only increase when stimulation increases.

13 The third nonspecific and unusually characteristic change in "stimulated" protoplasm is its increased ability to bind vital stains. As is well known, living cytoplasm and nuclei, under normal conditions, cannot be stained by acid dyes or basic dyes. The dye entering the cell usually concentrates in the form of newly formed granules or vacuoles, separate from the cytoplasm. The nucleus of a normal cell does not as a rule absorb vital stains**. However, this picture changes drastically as soon as

---

* For the complete list of papers on this problem, published before 1940, see Nasonov and Aleksandrov (1940). Here only the more important papers published by our team are given.

** Nuclei (macronuclei) of parasitic infusoria present in the alimentary tract of certain organisms are exceptions (Nasonov, 1932b).

the cell is subjected to a sufficiently strong stimulus. The ability of the cell to deposit the dye in the form of granules starts to decrease and finally is altogether lost*.

At the same time the cytoplasm, and especially the nucleus, which were colorless in the normal cell, begin to absorb the dye to a greater extent. Characteristicly the newly appearing structures in the nucleus stain most strongly, first the chromatin granules and later the membrane of the nucleus and nucleolus. The picture seen under the microscope closely resembles a fixed and stained histological preparation (Figure 1).

Such a preparation, therefore, should be considered as a picture of cells stimulated to the limit.

Among other nonspecific changes occurring in the stimulated cell are two of a more biochemical nature. Firstly, the well-known acid shift in the reaction of the cytoplasm and nucleus. Secondly, release and exit from the stimulated cells of various substances, for example potassium and phosphate ions, creatine, and some pigments. Simultaneously sodium and chlorine enter the cell. Release of electrolytes causes electronegativity on the surface of the cells.

The fact that above-mentioned features of "excited" protoplasm are all reversible in the initial phase is highly characteristic and important. Cessation of the stimulus causes an increase in colloid dispersion, which leads to the disappearance of the visible structures, and glowing in a dark field. The viscosity of protoplasm returns to normal. The increased ability of the living substrate to bind the dye disappears. The dye is released and passes into the surrounding solution, while the cytoplasm and the nucleus again become colorless. The potassium and phosphate ions that have left the cell are again absorbed and fixed by the protoplasm.

14 If the action of the stimulant is too prolonged, or if its intensity is too great, the changes in the protoplasm become irreversible and the cell dies. A large dose of any of the known stimulants causes death of the protoplasm. In this sense, any "excited" condition of the protoplasm may be considered as a stage leading to death. That is why in studying the changes induced in protoplasm by external factors, we suggested the term "paranecrosis", i.e., a condition near to death, but still reversible.. We used the term paranecrosis as opposed to "necrosis", which designates an already irreversible state of dying.

Vvedenskii (1901) suggested the term "parabiosis" to designate reversible, functional changes of the nerve in the area of application of various stimulants. This condition was characterized by a complex of nonspecific, reversible changes in excitability and conductivity of nerve fibers. He suggested that parabiosis may also occur in other tissues following the action of various stimulants on them.

Thus, if parabiosis means the sum of physiological or functional changes occurring in different cells in the region of the stimulants, paranecrosis should designate the sum of the changes in the living substrate itself and at their site of application. In our opinion parabiosis and para-
15 necrosis are but different aspects of the local reaction of the living system

---

* There are indications of a biphasic change in the ability of the cell to deposit dye granules following the action of stimulants. In a weak dose the stimulant sometimes enhances granule formation; on increasing the dose, the latter invariably ceases (Nasonov and Aleksandrov, 1940).

to environmental changes, and an outward manifestation of one of the basic properties of living matter—excitability.

It has already been mentioned that the nonspecificity of the local reaction is characteristic in that the same complex of changes appears in the cell following the action of the most varied stimulants. However, this statement is not absolute. Following the action of various stimulants there is undoubtedly a similar response, but distinguishing details still remain. For example, the most reversible type of paranecrosis is caused by narcotics; closest to it is paranecrosis due to temperature. The least reversible is that caused by ultraviolet and ionizing radiation.

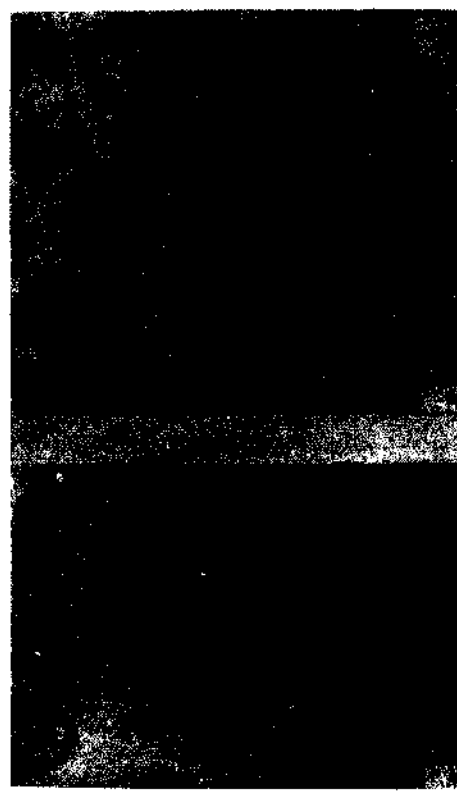


FIGURE 1. Epithelium of frog cornea. Stained with neutral red. Magnified 1260 times. (Photomicrograph by Kamnev)

a—normal; showing granules of neutral red, the nuclei are invisible and unstained; b—effect of dilute Ringer solution; stained nuclei are seen.

Thermal, electrical (cathode), and mechanical stimulants cause the appearance of paranecrosis immediately on application to the cells. In such cases it may be concluded that the changes occurred primarily due to the

stimulant. In other cases, a certain latent period may exist between the action of the agent and the appearance of paranecrosis, which may last many days. The latter is, for instance, characteristic of the radiation factor. It is obvious that in this case some other changes occur primarily, and paranecrosis is an aftereffect.

This also applies to agents directly interfering with metabolism. Thus, anoxia quickly causes paranecrosis (Nasonov, 1930; Aleksandrov, 1932; Makarov, 1934), the latter being a secondary change obviously due to a disturbance of normal metabolism.

Denaturation Theory of Excitation

The concept of paranecrosis enables many response reactions of the living substrate to be classified under the one heading "excitability", and endows this term with a concrete meaning. This implies that in all cases the various stimulants cause biologically and physico-chemically similar reactions.

What are these reactions?

In our monograph (1940) we analyzed our own data in detail, as well as data from the literature, and came to the conclusion that reversible changes in protoplasmic proteins, which are similar to the initial phases of denaturation of native proteins, form the basis for the paranecrotic reaction. It is not necessary to repeat in detail all the evidence in support of this as-
16 sumption, and those interested are referred to the monograph and to the more recent paper by V. Ya. Aleksandrov (1947). The main arguments are as follows:

1. The vast majority of proteins in living protoplasm can be denaturated. It is natural therefore to expect that living protoplasm, as a whole, also possesses this property.

2. The external features of denaturation of proteins produced by the cell are very similar to changes observed in protoplasm during paranecrosis. These are firstly loss of protein solubility, with a decrease in its dispersion and with resultant coagulation; secondly, increase in viscosity of denaturated proteins; thirdly, as in paranecrosis and in denaturation, a shift of the reaction in the acidic direction; fourthly, an increased ability of the protein to bind acid and basic dyes. This property was not known in the earlier literature, and was observed by us during our investigation of the increase in sorption properties of living protoplasm after excitation (Aleksandrov and Nasonov, 1939).

The latter aspect of both paranecrosis and denaturation is, in our opinion, important. Firstly, on this basis we could elaborate a method to estimate quantitatively the action of the stimulant on the tissue. Secondly, because a closer study of this phenomenon, as will be seen later, illustrates the processes occurring in living protoplasm after excitation. Therefore, the increase in sorption properties of living protoplasm and native proteins when acted upon by different agents will be discussed in detail in the following chapter.

3. It is important to note that agents causing denaturation of proteins are, at the same time, protoplasmic stimulants. Such stimulants include: a—increase in temperature; b—mechanical action (shaking or grinding in the dry state); c—radiant energy (ultraviolet and X-rays); d—audible sound;

e—increase in hydrostatic pressure; f—substances acting on the surface of cells; g—hyperacidity; h—changes in the ionic composition of the medium, etc.

4. The nonspecificity of the reaction has already been discussed. In regard to denaturation, agents of varied composition likewise cause a similar (i.e., nonspecific) change in protein. The proteins of the living cell are unique in their unified response to such varied stimulants. But at the same time, the nonspecificity of denaturation should not be regarded as absolute. Besides the common features of denaturation, certain differences may be found to be characteristic of the given stimulants.

5. Substances protecting proteins against denaturation also prevent the onset of paranecrosis; for example, sugars, polyhydric alcohols (glycerol, mannitol, etc.), neutral salts, and others.

6. Dehydration and drying increase the stability not only of living protoplasm, but also of native proteins in vitro.

17 7. A very important argument in favor of the similarity between denaturation and paranecrosis is the fact that particular properties, characteristic of a certain type of denaturation, are also characteristic of paranecrosis due to the same agent. For example, the same high temperature coefficient causes heat denaturation of proteins as well as thermal paranecrosis. In denaturation of proteins due to ultraviolet rays, x-rays, radium, a latent period and aftereffect are characteristic. The same latent period and aftereffect are also seen following the action of radiant energy on protoplasm.

A number of other common features in the local reaction of protoplasm to stimulants, and in denaturation of native proteins in vitro, will be discussed in different chapters of this book.

There are two objections to combining these two phenomena, i.e., that denaturation of proteins is an irreversible process, and that the physiological reaction of living protoplasm to excitation is in principle a reversible process.

In our monograph (Nasonov and Aleksandrov, 1940), extensive data from the literature show that heterodromous reversibility of denaturation is possible. In many cases, proteins denaturated by different means may revert to normal after comparatively simple treatment (for example, by a slight increase in pH). It appears that as long as the cell is alive and maintains its characteristic metabolism, it may "repair" the denaturation damage due to excitation. However, if the excitation is too strong and the normal metabolic mechanism is damaged, repair is impossible and the cell, or a part of the protoplasm, dies.

Also, there are indications that, in many cases, denaturation of proteins is reversible even homodromously, i.e., removal of the stimulus is sufficient to return the protein to its initial state. Thus, according to Anson and Mirsky (1934), trypsin, inactivated and made almost insoluble by thermal denaturation, spontaneously returns to the soluble form after cooling, with restoration of enzymatic properties.

Such homodromous reversibility was detected by Kunitz (1948), in relation to a trypsin inhibitor present in soil. After being heated to a high temperature, this protein becomes more insoluble, more accessible to digestion by pepsin, and loses its ability to combine specifically with trypsin. By merely cooling the solution of denatured protein, the latter returns spontaneously to its native state, in no way different from the initial

substance. Urea causes homodromous reversibility of many proteins. Dialysis of the urea from solution is sufficient to make the protein revert to its native state. The same homodromous denaturation is caused by sodium salicylate acting on hemoglobin (Belitser, 1950), etc. There is reason to assume that in the very early stages, any denaturation is homodromously reversible.

18 All these data force us to refute categorically any objections to the denaturation theory of excitation, which are based on the statement that any denaturation is in principle irreversible.

Another objection was that the strength or intensity of the effect, of a stimulant, in optimum dosage to produce excitation of protoplasm, is so much less than the intensity of the effect necessary for minimum denaturation of proteins in vitro, that these two phenomena are incomparable.

In our book, the inconsistency of this objection, too, is pointed out. The point is that the majority of native proteins (a favorite material for biochemists) are quite stable. They may exist for a long time at room temperature, and in most cases originate not from cell protoplasm, but from either the basic substance of the connective tissue (plasma), or from cell secretions (egg protein). Also in this group are proteins such as serum albumin or serum globulin, egg albumin or egg globulin. For these proteins, the temperature of denaturation (55-60° C) is considerably higher than the temperature "stimulating" living cells (35-40° C). However, the denaturation temperature for a cellular protein like myosin coincides with the "stimulating" temperature of the muscle from which it was obtained. Thus, Mirsky (1938) showed that gradual heating of the sartorius muscle of the frog caused it to contract suddenly at 38° C. In his opinion, this contraction was entirely physiological, since it was freely reversible, and should be considered as a physiological response reaction of the muscle to thermal stimulation. At exactly this temperature the manifestations of paranecrosis appear (see below). Mirsky further showed that myosin from frog muscle heated to 38° C did not undergo sharp external changes but if the temperature was kept at 30° C for several minutes, almost all of the myosin became insoluble.

It is interesting to note that in rabbits, heat contraction of muscles starts only at 45° C. Likewise, the first signs of denaturation of the extracted myosin also occur around 45° C*.

From the above example it will be seen that if certain precautions are taken, protoplasmic proteins may be subjected to a denaturation temperature very close to that causing excitation of the cell and paranecrosis.

The second argument (based on the idea that the threshold value for stimulant doses and the threshold value for doses necessary for denaturation of native proteins in vitro are quantitatively not comparable) can also be rejected on the basis of the above data.

---

* Komkovaya and Ushakov (1955), and Ushakov (1956), succeeded in showing that the heat resistance of certain tissues is determined by the heat resistance of their proteins.

Chapter 2. Quantitative Evaluation of the Degree of Alteration of Cells by the Action of Stimuli

Increase in Sorption Capacity of Native Proteins Due to the Influence of Denaturating Agents

In the previous chapter it was stated that one of the most characteristic features of paranecrosis was the increased capacity of living protoplasm and the nucleus to bind vital stains. On the basis of a number of facts it was concluded that denaturation of cell proteins was the foundation of paranecrosis. However, in the biochemical literature, there were no indications that the sorption capacities of native proteins increased on denaturation. In order to test this assumption, Aleksandrov and Nasonov (1939) performed a series of experiments with native proteins.

Serum was placed in collodion sacs which retained the proteins. The sacs were submerged in solutions of dyes (in Ringer's solution). After diffusion, equilibrium was established in each case between the sac contents and the surrounding solution of the dye and the solution was poured from the sac into a test tube. The dye was extracted by acidified alcohol and determined colorimetrically. In this manner, the amount of dye bound by a unit of dry weight of protein was determined.

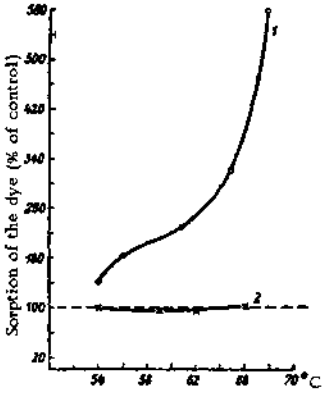


FIGURE 2. Binding of vital stains by horse serum proteins previously subjected to half-hour treatment at different temperatures (from Aleksandrov and Nasonov, 1939)

1—methylene blue (basic dye); 2—cyanol (acid dye).

Figure 2 illustrates the binding of methylene blue (0.001%) by horse serum proteins previously subjected to half-hour treatment at different temperatures. Heat denaturation at 54° C led to an increase in sorption of the

dye by 40%*. The sorption curve continued to increase and, starting at approximately 60° C, rose steeply, to reach 580% of the initial value within the range of 68° C.

It follows from Table 1, which illustrates data from experiments performed by the same method, that sorption of methylene blue and other basic dyes increases considerably after denaturation. Increase of sorption to 200% is also observed after denaturation by alcohol, acetone, and ultraviolet light. In this respect, temperature is no exception. In nondenaturated serum, proteins bind the same amount of acid dyes (cyanol, trypan blue) as denaturated proteins. Thus on denaturation the sorption capacity of these proteins increases only in relation to basic dyes.

20

Table 1

Sorption of dyes by horse serum proteins subjected to prior denaturation by different methods (from Aleksandrov and Nasonov, 1939)

Method of denaturation	Dye	Sorption by denaturated serum (% of sorption by native serum)
Temperature 66° C	Toluidine blue, 0.001 % (basic)	208
" 63° C	Brilliant cresyl blue, 0.001 % (basic)	174
" 59° C	Trypan blue, 0.004 % (acid)	101
" 66° C	Cyanol, 0.002 % (acid)	103
Ultraviolet irradiation for 65 min	Methylene blue, 0.001 % (basic)	105
Ultraviolet irradiation for 2 hrs 30 min	The same	135
Ethyl alcohol	" "	197
Acetone	" "	191

Table 2

Sorption of dyes by chicken proteins subjected to heat denaturation (59° C) (from Aleksandrov and Nasonov, 1939)

Dye	Sorption by denaturated serum (% of sorption by native serum)
Methylene blue, 0.001 % (basic)	139
Toluidine blue, 0.001 % (basic)	125
Cyanol, 0.002 % (acid)	1228
Trypan blue, 0.001 % (acid)	1643

---

* It is interesting that denaturation of serum by heating for half an hour at 54° C did not cause any turbidity. Thus, by increase of the sorption of dyes, alterations in native proteins may be detected earlier than by turbidity.

There are probably specific differences connected with the special properties of different proteins. Experiments performed on chicken pro-
21 teins using the above technique have shown that after denaturation, their capacity to bind both basic and acid dyes increases (Table 2). The increase in the latter (1228 and 1643 %) is many times greater than the former (139 and 125 %). Chicken protein is a better model than horse serum proteins for reproducing the given property of living protoplasm.

The property of proteins to increase their binding capacity for dyes after denaturation was later confirmed in our laboratory by Braun (Braun, 1948a, 1948b, 1949, 1951; Braun and Feldman, 1949; Braun, Savost'yanova, and Morozova, 1950) using pure protein preparations. In order to determine the fate of the dye bound by the protein, different methods were used. Results were obtained partly by measuring the diffusion rate of free dye from a mixture with the protein in gelatin gel. A method was also elaborated to determine the affinity of the dye to the protein, based on the absorption spectra of (a) solutions of dyes, and (b) mixtures of the dyes with proteins. The method was based on the following observations. Absorption curves of Congo red, Nile blue, and other dyes in salt solutions (in contradistinction to aqueous solutions of the same dyes) are shifted by 10-40 m μ in the direction of the short waves, and the absorption intensity is diminished. If a protein is added to a solution of dye whose absorption-spectrum is changed by the addition of a neutral salt solution, the absorption spectrum shifts in the direction of the long waves, and the absorption intensity increases. Thus, the absorption curve is, as it were, "corrected", approaching the curve of the aqueous solution of the dye. This phenomenon is explained by interaction between the protein and the aggregated forms of the dye, by binding the released valencies of the dye to the protein. The degree of "the corrective effect" is a measure of reactivity of the proteins. The "corrective effect" of denaturated proteins is invariably manifested to a much higher degree than that of native proteins (Figure 3). Using this method, it can be quickly and convincingly shown that protein reactivity increases denaturation.

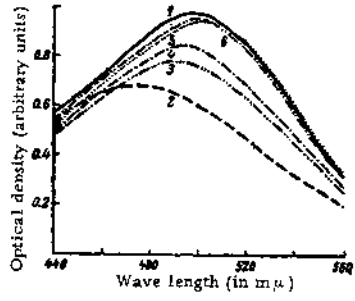


FIGURE 3. Effect of native and heat-denaturated egg albumin on the absorption of spectrum of Congo red, modified by the addition of a neutral salt solution (from Braun, 1949)

1, 2—absorption of Congo red solutions ($3 \cdot 10^{-5}$ M) in (1) water and (2) in 0.5 M KCl; 3-6—absorption of Congo red solutions in 0.5 M KCl upon addition of egg albumin (0.4 %); (3), native, (4), heated at 45° C, (5) at 59° C and (6) at 63° C.

In studies of the interaction between proteins and triphenylmethane dyes (malachite green, crystal violet, and others), it was observed that these dyes turned colorless in the presence of myosin and certain other
22 native proteins. It has been shown that the discoloration was accompanied by binding of the dyes to the proteins through the sulfhydryl groups of the latter. This mechanism is similar to the carbonyl transformation of dyes, and indicated the possible formation of covalent bonds between proteins and dyes. After denaturation of protein, its ability to decolorize triphenylmethane dyes increased considerably.

Finally, the results of experiments with myosin fibers from rabbit muscle are especially instructive. The fibers were stained by basic and acid dye solutions in both their native state and after denaturation by high temperature or urea. It was obvious even to the naked eye that after denaturation the sorption of the dye increased considerably. This was especially noticeable with the central portions of the fibers heated for 3 minutes to 45° C (Figure 4, A). On staining with triphenylmethane dyes, the heated parts of the fiber became colorless (Figure 4, B). In order to obtain a quantitative estimate of the increase in sorption, the dye was extracted from fibers stained under standard conditions, with acidified alcohol. The extract was measured in the photometer. The amount of dye bound by the denatured fibers was expressed as a percent of the amount of dye bound by untreated fibers (Table 3).

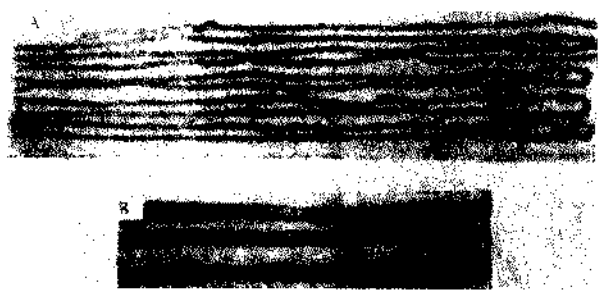


FIGURE 4. Myosin fibers stained with (A) Congo red and (B) light green. The central part was denaturated by heating prior to staining. (A—from Braun, 1949; B—from Braun, 1948b).

The ability of various proteins to bind basic and acid dyes in solutions was studied. A series of native proteins was also studied in this respect. Figures 5 and 6 show that prior heat treatment of proteins considerably increased the sorption of both acid and basic dyes. However,
23 this increase may proceed at unequal rates. It follows from Figure 6 that increase in temperature (to approximately 30° C) caused an increase of sorption of the basic dye (methyl violet). Later, starting from 38° C, the sorption of the acid dye (cyanol) increased.

The affinity of proteins to dyes and the amount of sulfhydryl groups liberated upon denaturation were measured. The appearance of turbidity

in the solutions and their viscosity were observed. It was found that the increase in the sorption capacity of protein, as contrasted with the increase in the number of SH-groups and changes in turbidity and viscosity, occurred with a weaker denaturating effect. This result confirmed that not all the results of denaturation do appear simultaneously. The increase in the nonspecific reactivity of protein may be noticed at the early stages of denaturation; it is a sensitive index of the changes taking place in the protein.

Table 3

Effect of denaturation on binding of dyes by native and denaturated myosin fibers (from Braun, 1948b)

Denaturated by		Amount of dye bound by the denatured fibers (% of the amount of dye bound by native fibers)				
		Neutral red	Methylene blue	Nile blue	Congo red	Cyanol
Heating for 10 min at:	37° C	119	126	139	156	136
	45° C	134	158	170	204	154
	60° C	144	168	186	210	164
Action of urea:	2 min	116	124	122	134	106
	5 min	153	160	151	179	139

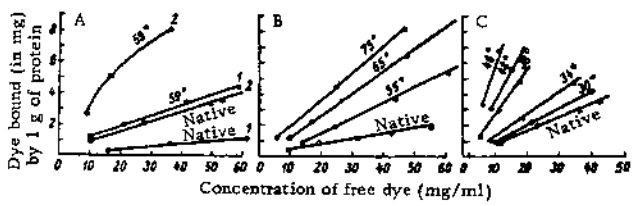


FIGURE 5. Binding of dyes by native and denaturated proteins (from Braun, 1948b)

A—binding of (1) methyl violet and (2) trypan blue by albumin (native, and denaturated by prior heating); B—binding of methyl violet by egg globulin (native, and denaturated by prior heating); C—binding of methyl violet by myosin (native, and denaturated by prior heating).

Haurowitz, Di-Moia, and Tekman (1952) later described an increase in the binding of an acid dye (Congo red) by egg albumin, denaturated by high
24 temperature*. Congo red, at pH 2, has a dark-blue color. In the presence

---

* These authors were probably not familiar with our work.

of native proteins, this color is retained. In the presence of denaturated proteins it turns red, the color of adsorbed Congo red molecules. This enables denaturation to be determined by a spectrometer. It has been shown that binding of Congo red after denaturation of egg albumin at 40° C and 100° C considerably increases. The peak of the absorption spectrum shifts to the right from 496 to 510 mμ). However, not only dyes are more strongly bound by denaturated proteins. Fischer (1935) has found that denaturated chicken protein shows increased binding of heparin, a monobasic organic acid. Figure 7 shows how increase in temperature enhances this process in steplike fashion.

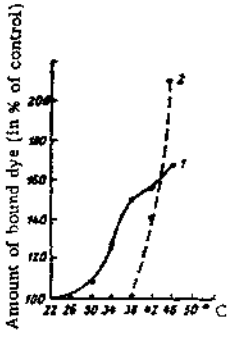


FIGURE 6. Binding of (1) methyl violet and (2) cyanol, by myosin denaturated by heating (from Braun, 1948 a)

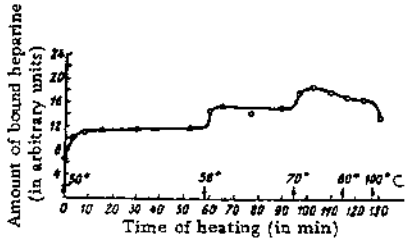


FIGURE 7. Effect of heat denaturation on binding of heparin by protein (according to Fischer, 1935)

Temperature changes are marked by arrows

Pasynskii, Volkova, and Blokhina (1955), and Pavlovskaya, Volkova, and Pasynskii (1955), confirmed our data on the increase in sorption capacity of proteins after denaturation. They investigated the binding of labeled methionine by pure preparations of human serum albumin, whole chicken egg white, and whole serum. Denaturation was performed by the following methods: high temperature, shaking, ethyl alcohol administration, and ultraviolet and x-ray irradiation. The results are shown in Table 4.

The data in Table 4 indicate that all the denaturation methods tested by the authors resulted in a significant increase in binding of methionine.

We have earlier observed (Nasonov and Aleksandrov, 1940, p. 160), that the sorption method is superior to others, since by its use denaturation by weak agents can be detected. This was confirmed by Pasynskii et al. (Pasynskii, Volkova, and Blokhina, 1955, p. 319): "In this manner (i.e., sorption), very slight denaturation changes, for example due to heating for 20 minutes at 37° C, ultraviolet irradiation for 1 minute, x-ray irradiation with a dose of 2,000 r, and other methods, may be detected.

The reason for increased sorption (by denaturated proteins) of organic electrolytes, e.g. dyes, aminoacids, and heparin, is not fully understood.

According to contemporary theories (Belitser, 1950; Putnam, 1953, and others), no splitting of the protein molecules or addition to them of any new radicals or groups takes place on denaturation. The basis of denaturation is unfolding of the polypeptide chain forming the protein molecule. As a result of this the protein molecule loses the usual spatial configuration of the characteristic parts of the complex chain, which determines its specific properties*. After unfolding of the globule, active chemical groups appear at the surface of the molecule, which in the native protein were inside the globule in a latent and inactive condition.

With formation of these groups, protein molecules interact. Large aggregates form, which precipitate from solution (Haurowitz, 1936). This explains the decrease in the degree of dispersion of protein solutions after denaturation, and the increase in their viscosity caused by deformation of globular molecules. These form elongated or even threadlike structures (the appearance of streaming birefringence). On denaturation, the active groups on the surface of protein molecules, positively or negatively charged, may bind acid and basic dyes, aminoacids and other compounds, also bearing positive or negative charges (Haurowitz, 1936).

26 The groups appearing at the surface of the molecule are polar, and represent ends of side chains (Putnam, 1953). The best investigated are the easily-detected sulfhydryl groups (SH). It does not follow, however, that the appearance of these groups is of greater importance than the formation of other radicals after denaturation. Nevertheless, an increased number of SH-groups is often considered an index of denaturation.

Table 4

Effect of denaturation of serum albumin on adsorption of labeled methionine (according to Pasynskii, Volkova, and Blokhina, 1955)

Treatment of preparation	Activity (c.p.m. per 10 mg of protein precipitate)
Initial protein	50
Denaturation:	
by heating at 37°C (20 min)	150-200
" " at 100°C (20 min)	400-650
by shaking (18 hrs)	250
by ethyl alcohol administration (5:1)	120
by ultraviolet light (1 hr)	300
" " " (1 min)	86
by x-rays (10,000 r)	160
" " (2,000 r)	70

---

* Certain investigators recently broached the idea that no great changes take place in the configuration of protein molecules after denaturation (Belitser, 1955; Pasynskii, 1955).

Since the discovery of the appearance of sulfhydryl groups after denaturation of egg albumin (Heffter, 1907; Arnold, 1911), many suggestions have been made as to the nature of this reaction. The point is that many proteins show the presence of reactive SH- or other polar side-groups, the number of which does not correspond to their analytical composition. However, after denaturation, the number of these groups increases considerably. Therefore, the groups were called "hidden", "masked", "inaccessible", etc. It is not clear whether they were masked only by a spatially folded chain of native protein, by intramolecular hydrogen bonds, or in any other way. Their appearance after denaturation is gradual, not sudden, and takes place not necessarily parallel to an increase in viscosity (Putnam, 1953, p. 831). The appearance of these groups is of special interest in connection with the work of Koshtoyants et al. (1951, 1952).

Among other reactive groups formed after denaturation of proteins are the disulfides (S-S) discovered by Walker (1925), and phenol and indole which, like SH-groups, may exist in a masked state in native protein, and be detected only after denaturation.

Finally, of special interest is the appearance of carboxyl and amino-groups. According to our theory, the increase in binding of dyes is due to these radicals.

Until recently it was thought that on denaturation no unmasking of NH_2- and COOH-groups occurs, since the titration curves of native proteins do not differ from those of denatured ones. In the case of carbonyl hemoglobin (Steinhardt and Zaiser, 1951a and 1951b), NH_2-groups in serum globulins, and beta-lactoglobulin (Porter, 1948) it may also be possible to detect carboxyl group. Porter showed that if NH_2- groups in these proteins are determined by acetylation with acetic anhydride, or by reaction with ketene, these groups can be fully detected. If, however, they are determined by the use of the FDNB reagent (1:2:4 fluorodinitrobenzene), this reagent detects only a part of the NH_2- groups in native proteins. After denaturation, all these groups can be detected by the use of the same reagent. Porter's explanation is that in the native state, parts of the NH_2-groups exist within a densely folded peptide chain, and are inaccessible to such large molecular compounds as FDNB, although accessible to smaller molecules.

Haurowitz and his co-workers (Haurowitz, Di-Moia, and Tekman, 1952) likewise explained the increase in staining power of proteins by Congo red. In their opinion, the low reactivity of native proteins is due to
27 the inability of certain groups to react within the densely folded threadlike chain.

According to the above authors, the question of whether or not denaturation causes an increase in the activity of cationic groups cannot be solved by the use of electrometric titration, since all protein groups are accessible to such small ions as hydrogen. However, large ions, such as those of the dye, cannot penetrate the molecule of native protein, due to the densely folded peptide chain. Therefore it may react only with the more accessible groups on the surface of the molecules.

In our opinion, all the polar groups of protein molecules released by denaturation may strengthen the binding of dyes, aminoacids and other organic electrolytes. Therefore we do not agree with Pasynskii, in whose opinion in this respect the SH-groups are more important, on the grounds that addition of cysteine weakens the effect of increased binding upon denaturation. The point is that cysteine, as an aminoacid, may not only

specifically block SH-groups, but also combines with other active groups, thus interfering with the binding of labeled methionine. Furthermore, after denaturation, not only does the binding of basic dyes increase, but also that of acid dyes. Both these reactions cannot be due to SH-groups.

All these biochemical investigations are especially interesting to physiologists, since they give a clear picture of the changes occurring in living protoplasm when acted on by stimulants. They also explain the increase in staining power after excitation and damage.

Method for Determining the Extent of Alteration of Living Protoplasm by the Action of Stimuli

The previous chapter reviewed the nonspecific changes in protoplasm which occurred after cellular excitation, as detected by an increased capacity to bind vital stains. These changes were termed paranecrosis (Nasonov and Aleksandrov, 1940). However, the method of vital staining led to a false qualitative distinction between the normal and the "excited" cell.

Later, using the method of quantitative colorimetric determination of the degree and severity of paranecrosis, evolved in our laboratory by Braun and Ivanov (1933), it was found that there were not two separate states (normal and paranecrotic) and that furthermore the reaction of living protoplasm to stimulants was very gradual, i.e., the strength of excitation was proportional to the change in the protoplasm. It was true that in certain cases, there was a sharp increase in the response, which approached more of a threshold value. However, under these conditions, we had never observed a sudden change sufficient to justify the term "all or none" response*.

Our method of investigating the degree of change of protoplasm was to study its ability to bind stronger dyes after excitation. The method was as follows: control and experimental tissues were placed, for the same length of time, in dyes diluted with Ringer's solution, the experimental tissue being subjected to various stimulants. Both sections of tissue were then removed from the solution, rinsed in distilled water, and placed into separate test

---

* In the biochemical literature opinions are divided whether the change in protein is sudden or not. Some authors believe that the protein molecules may exist only in two extreme states, native or completely denaturated. The proponents of the "all or none" theory explain the transition of the protein by the presence in solution of a variable percentage of completely denaturated molecules (see Belitser, 1950). Others believe that in each protein molecule the unfolding of the molecular configuration may take place gradually after denaturation, passing through a number of intermediary stages (for recent literature, see Putnam, 1953). This problem has not yet been completely solved, however the majority of authors are against the "all or none" principle. To the physiologist studying the processes of excitation, the exact explanation of the change is inconsequential.

tubes containing a small, accurately measured amount of 70% ethyl alcohol, acidified with 2% sulfuric acid. After 24 hours practically all of the dye had been extracted by the acidified alcohol, after which the stained alcohol from the experimental and control tissues was examined either photometrically (using a Pulfrich comparative photometer) or with a sensitive electrophotometer. The amount of bound dye from the experimental tissue was usually expressed as a percentage of that from the control.

To obtain reliable results by this method, the arithmetic mean of not less than 10 single experiments was used. The data were considered statistically significant only when the arithmetic mean was three times higher than the square of the mean error.

If the experimental and control tissue sections were of different dimensions, corrections were made for weight. After extraction of the dye with alcohol, the sections were dried to constant weight.

For vital staining different stains were used. The basic dyes most often used were neutral red and sometimes methylene blue. The latter was less convenient since at low pH methylene blue may be reduced in the tissues, giving the colorless leukoform.

29 As shown by Troshin (1951c), concentrated solutions of neutral red (Figure 8) are most convenient to detect differences between living and damaged tissues. However, concentrated solutions may be toxic for the tissues, therefore solutions of neutral red somewhat below the threshold of toxicity are recommended. We normally used solutions of 0.1 to 0.01%

Neutral red easily precipitates from solution in the presence of carbonate. Ringer's solution without carbonate was used.

The staining time varied from 10 to 30 minutes, depending on the size of the tissue. Staining of tissues from cold-blooded or warm-blooded animals was usually performed at room temperature (18-20° C), since destruction and death of tissues of warm-blooded animals in the isolated state takes place more rapidly at body temperature (37-40° C). With an alkaline shift in the medium, there ought to be an increase in staining power with basic dyes and a decrease with acid dyes. However, following excitation, an increased staining power with both basic and acid dyes was observed. Thus in studies of this kind, the use of both groups of dyes is recommended.

The acid dyes normally used were a 1% solution of cyanol, or a 0.03% solution of phenol red. Living tissues stain much weaker with acid than with basic dyes. The alcoholic extracts* are therefore very pale and have to be measured in horizontal microcuvettes.

However, in the case of tissues damaged by various agents, the acid dyes have a greater staining power than basic dyes. Obviously, the number of free positively charged radicals at the surface of the molecules of living protoplasm which may fix acid dyes is extremely small, and they appear only upon excitation and damage of the tissues (Figure 8).

The main drawback to our method is that the dyes diffuse very slowly into living tissues. Diffusion equilibrium is not completed within the staining time (10 to 30 minutes).

30 Figures 88 and 89 (see page 131), from the paper by Troshin (1956), show that diffusion equilibrium between the medium and the sartorius muscles of the frog is established after two to three hours. Only in a few cases where

---

* For extraction of phenol red, 70% alcohol is used, 5 ml of concentrated H_2SO_4 being added to 100 ml of the alcohol solution.

diffusion equilibrium is quickly reached can the binding of dye be expressed per unit of tissue weight. In the majority of cases this formula is not valid.

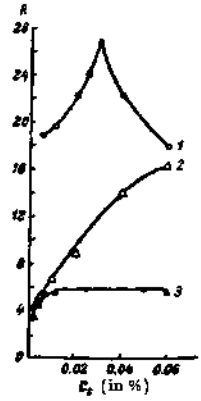


FIGURE 8. Relationship between the staining power of dead and living frog muscles (R), and the concentration of dye in the surrounding solution (C_S). (According to Troshin, 1951c)

1—phenol red; 2—neutral red; 3—rhodamin.

The most suitable materials for this method are tissue suspensions where the equilibrium between the dye in the medium and the dye in the cell is reached within 5-10 minutes. Raevskaya and Troshin (1937) used a suspension of spermatozoa, and Romanov (1951) a suspension of yeast cells.

When used with cell suspensions, this method is applied by the following procedure. The cell suspension is accurately divided into two equal parts. and placed in separate test tubes. Both tubes are simultaneously centrifuged and decanted, and equal amounts of the dye in Ringer's solution added. Both test tubes are then shaken. The contents of one tube is used as a control, the other is tested against various agents. The test tubes are again centrifuged, the centrifugate is washed with distilled water and again centrifuged. After decantation, acidified alcohol is added in order to extract the dye. The extracts obtained are measured colorimetrically. Here, too, the results may be expressed either as a percentage of the control or in absolute numbers relating the dye to a unit of dry weight. In the latter case, after extraction of the dye, the precipitate should be dried and weighed.

Sometimes suspensions of tissue fragments or even whole organs are used. In this case, it is advisable to use paired organs, which are easily accessible and taken from the same animal, so that one may serve as a control for the other. For this purpose the sartorius muscles of the frog, semitendinosus muscles of mammals, spinal ganglia of the frog and of laboratory mammals, paired nerves (sciatic), frog kidneys, etc. are very convenient.

In the preparation of organs care should be taken to remove all fragments of damaged tissues, since they stain strongly and may lead to considerable errors.

In studies using the sartorius muscles of the frog, threads are tied to their ends (to the tendon and to the pelvic bone to which the muscle is attached), and the muscles are thus tied to a bent glass rod (Figure 9). The muscles should be straightened but not stretched. All procedures, including staining, are performed with the muscle tied to the rod. After staining, the tendon and bone are excised and the released muscle is placed in acidified alcohol to extract the dye.

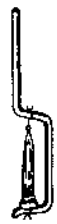


FIGURE 9. Sartorius muscle of the frog tied to a glass rod

In this experiment it was difficult to decide how long to expose the tissue to the various stimulants. Theoretically the tissue should be stained
31 during excitation, but this is frequently impossible, since the majority of stimulants themselves modify the staining process.

The following factors modify the staining of living or dead tissues: temperature; change in pH of the medium; addition of various substances to the solution, especially of electrolytes. In the latter case the greatest decrease in staining occurs in the region of biologically active concentrations of the added substance (Nasonov and Aleksandrov, 1937). In studying the action of temperature or chemical substances, it is only possible to use the staining method immediately after removing the stimulus (cooling, rinsing, etc.). Consequently, in a number of cases it was not the direct action of the agents which was studied, but the resulting changes occurring after their action ceased.

The results of such a series of experiments are illustrated in Figure 10. The abscissa shows the logarithms of the molar concentrations of different substances. Living frog muscles were kept in solutions of these (in Ringer's solution) for one hour and 30 minutes, after which time they were thoroughly washed in Ringer's solution for one hour and stained for one and a half hours in 0.01 % methylene blue. Subsequently the dye was extracted with acidified alcohol, and the extract measured colorimetrically. The ordinate shows the staining intensity of the experimental muscles as compared with the controls, the staining of which is considered as 100 %. The narcotic concentrations are given in vertical straight lines. Noticeable increase in staining starts at those concentrations of the investigated substances which cause narcosis in the muscles.

32 There are stimulants which, when applied to non-living objects, do not themselves affect the degree of staining, for example high pressure, sonic waves, irradiation, etc. Their effect on protoplasm may be studied by the method of vital staining. This group may also include physiological stimulation of nerve cells and nerve and muscle fibers.

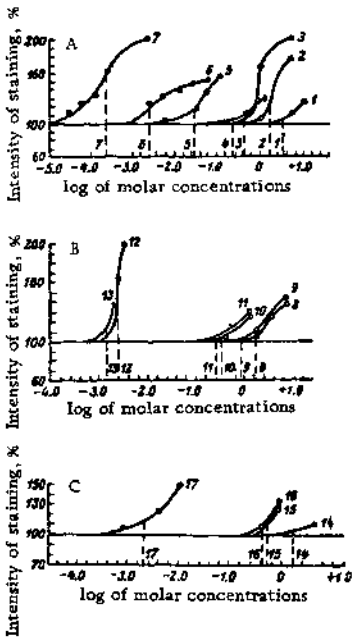


FIGURE 10. Effect of various substances on the staining power of living frog muscles (according to Nasonov and Aleksandrov, 1937).

A: 1—methyl alcohol; 2—ethyl alcohol; 3—propyl alcohol; 4—butyl alcohol; 5—chloral hydrate; 6—caffeine; 7—saponin. B: 8—urea; 9—acetone; 10—glucose; 11—saccharose; 12—quinine; 13—optaquine. C: 14—glycerol; 15—ethyl alcohol; 16—urethane; 17—carbon disulfide. The narcotic limit concentrations of these substances are given in vertical lines.

Another drawback to the use of the above method is the formation of granules. It has already been mentioned that in normal, living cells the protoplasm and nucleus hardly stain at all. The dye that penetrates the cell accumulates in the region of the intracellular Golgi apparatus in the form of granules (Nasonov and Aleksandrov, 1940). In a number of convincing studies, it has been shown (Feldman, 1948a, 1948b, 1950, 1953) that the process of granule formation may be looked upon as a complex coacervation of two colloids—of a certain protein component of the protoplasm, and the dye solution. After protein denaturation, the process of coacervation is

disturbed, which is most probably the reason why granule formation stops in excited cells.

However, after extracting the dye with alcohol, not only the diffused dye, but also that deposited in granules is extracted from the cells.

The latter predominates in resting cells, the former in "excited" cells. Intensive granule formation was a source of error in the results obtained by Krasilnikova (1954). To avoid this source of error, staining time should be shorter and stronger solutions of dyes should be used. In muscles, for example, stained with 0.1% neutral red for 10-30 minutes, there is practically no granule formation, and on microscopy only diffused staining is observed. However, when the muscle fibers are kept in the dye over 1-2 hours, very small and later increasingly larger granules of dye appear.

Acid dyes are not deposited as granules under the above conditions, and therefore do not cause this source of error

The direct effects of sound waves and high hydrostatic pressure on living cells will now be considered. These effects are of special interest, since they are caused by the few agents whose effect on the protoplasm, as already mentioned, may be studied by vital staining during the application of the stimulus.

Audible Sounds as Direct Stimuli of Cellular Protoplasm

We are not aware of any reports in the physiological literature where the effects of such stimuli have been studied. It is usually assumed that the sound stimulus is perceived exclusively by the ears, and that in the organ of Corti sound is formed, in some manner, from vibrations of the tympanic membrane acting on the cells of the auditory epithelium as mechanical
33 stimulus. However, it may be assumed that protoplasm also receives sound vibrations directly, without the participation of the auditory organs or the nervous system. We reached this conclusion on the basis of the denaturation theory of excitation.

As already mentioned, agents causing denaturation of proteins ought to be stimulants of living protoplasm. From this point of view the interesting paper by Chambers and Flosdorf (1936) deserves attention. These workers showed that sound waves of high intensity (given off by sirens for underwater signalling) may cause denaturation of native proteins in vitro. According to their data, a direct transformation of sound energy into chemical energy occurred. It may therefore be justifiable to assume that sound waves may be also directly received as a stimulus by living protoplasm without being transformed into a mechanical or tactile stimulation via the organ of Corti.

These considerations were the basis of a study performed in our laboratory (Nasonov and Ravdonik, 1947). The source of sound was an electro-acoumetric device which produced sounds of different frequencies, from 100 to 10,000 cps, with various levels of sound intensity from 0 db to 120 db.

The sartorius muscle of frog was used. The amount of dye bound by the muscle was determined by a colorimeter. Paired muscles were attached to bent glass rods by tight threads (Figure 9) and submerged in two Petri dishes containing the dye, prepared in Ringer's solution. One muscle served

as a control; the other was placed on a soft pad, and covered by the sound diffusor in such a manner that the center of the diffusor was 15 centimeters from the muscle. The Petri dish did not directly touch the reproductor. A sound was initiated, and continued during the whole time of staining, i.e. 20 minutes. The two muscles were simultaneously removed from the dye solution, rinsed in fresh Ringer's solution, examined under the microscope, and transferred to two test tubes with 5-10 ml of 70% alcohol in each, acidified with 2% sulfuric acid. The extracts of the experimental and control muscles were measured colorimetrically, the staining intensity of the experimental muscle being stated as a percentage of the control.

The effect of sound on the vital staining of muscles by a basic dye—0.1% solution of neutral red, and by an acid dye—1% solution of cyanol, was studied.

The experimental error of the method was first established empirically. This depended on the inaccuracy of measurements and on individual differences between two paired muscles. For this purpose, paired muscles were stained without sonic stimuli; the muscle of the right side was always the experimental, and the left the control. The results obtained are given in Table 5, from which it follows that maximal individual deviations reached 9%, while the arithmetical mean of deviations from 10 experiments equalled 2.4 ± 1.0%. Consequently, the method used allowed for an error within the
34 indicated limits, if for each point of the curve an arithmetic mean of 10 separate experiments was used.

Table 5

Staining differences between the right and left sartorius muscles of a frog (according to Nasonov and Ravdonik, 1947)

Experiment	Change in staining, %
1	+ 3.0
2	- 3.0
3	- 1.0
4	+ 4.4
5	- 1.6
6	- 4.7
7	+ 4.0
8	+ 8.0
9	+ 9.0
10	+ 6.0
Arithmetic mean	+ 2.4 ± 1.0

Later the effect of sound on nonliving substrate was studied. Paired muscles were killed by immersion for 5 minutes in Ringer's solution heated to 90° C. One was used as a control, and the other exposed to sound of 3,000 cps at an intensity level of 94 db. Both were then stained. The results are given in Table 6, from which it will be seen that even if sound has an effect of staining of dead substrate, this effect is very small (+3.3%). This weak positive result may be explained by the fact that treatment of muscle with hot (90° C) Ringer's solution for 5 minutes did not completely denaturate the muscle protein.

Table 6

Changes in staining power of sartorius muscles following stimulation by sound (in frogs killed by high temperature) (according to Nasonov and Ravdonik, 1947)

Experiment	Change in staining, %
1	- 1.0
2	+ 5.2
3	+ 4.0
4	+ 1.0
5	- 2.0
6	+ 0.7
7	+ 2.4
8	+ 7.0
9	+ 10.0
10	+ 6.0
Arithmetic mean	+ 3.3 ± 1.1

After these preliminary experiments, the effect of sound waves of 200, 500, 1,000, 2,000, 3,000, 4,000, 5,000, 7,000 and 10,000 cps was studied in experiments with neutral red, and that of 100, 3,000 and 10,000 cps in experiments with cyanol in all cases of living muscles. All the sounds were of similar intensity, about 94 db*. Each series consisted of 10 experiments. In Tables 7 and 8 only the arithmetic mean of each ten experiments and the calculated mean square errors are given. It will be seen that the mean differences are more than 3 times greater than the mean square errors. In certain cases, they are more than 5 times greater. Thus, the data are statistically significant.

Table 7

Increase in staining power (with neutral red) of sartorius muscles of frog, stimulated by sounds of different frequency, at the same level of intensity—94 db (according to Nasonov and Ravdonik, 1947)

Frequency (cps)	Arithmetic mean and mean square error (%)	Frequency (cps)	Arithmetic mean and mean square error (%)
0	+ 2.4 ± 1.0	3,000	+ 28.3 ± 5.0
200	+10.8 ± 2.5	4,000	+ 20.5 ± 4.1
500	+17.3 ± 3.3	5,000	+ 19.0 ± 3.6
1,000	+19.7 ± 6.2	7,000	+ 17.0 ± 3.2
2,000	+22.8 ± 4.0	10,000	+ 13.9 ± 3.3

---

* The level of sound intensity decreased considerably on passing from air to Ringer's solution.

The data from Tables 7 and 8 are shown in Figure 11, 2, 3, from which it follows that the sounds at all frequencies used caused a noticeable reaction in muscle protoplasm, with an increase in staining of both basic and acid dyes.
35 However, the magnitude of this reaction was not always the same. At 200 cps it was minimal (10.8% for neutral red and 12.7% for cyanol), but gradually increased up to 3,000 cps. Here a maximum was clearly observed, after which the intensity of staining power gradually decreased with increase in frequency.

Table 8

Increase in staining power (with cyanol) of sartorius muscles of frog, stimulated by sounds of different frequencies at the same level of intensity—94 db (according to Nasonov and Ravdonik, 1947)

Frequency (cps)	Arithmetic mean and mean square error (%)
0	0
200	+12.7 ± 1.7
3,000	+39.0 ± 4.5
10,000	+15.1 ± 2.4

Curve 3 (1% cyanol) is generally similar to curve 2 (basic dye) but all its points are situated higher.

The effect of sounds of different frequency on muscle sorption is easily shown. At first, curve 2 was obtained. Using spring-summer frogs in 1946, Ravdonik (1949) repeated the experiment, using the same method with autumn-winter frogs. He obtained curve 1 (Figure 11), which was a reproduction of curve 2, but with all its points located below those of the latter, probably due to lower reactivity of autumn frog's muscle.

Thorough microscopy of stained muscle, stimulated by sound reproducing a maximal reaction (3,000 cps), did not disclose any noticeable structural changes or vital staining of nuclei, except for an evenly diffused staining of the protoplasm, differing from the control only in intensity. As in the experiment, no granular deposits were observed in the control.

The effects of sound as a muscle stimulant were further studied by the following method: an isolated sartorius frog muscle was connected by a thread to the recording lever of a myograph. The diffusor of the sound
36 apparatus was placed 15 centimeters away. The sound stimulus lasted 5-10 minutes. The end of the time interval was recorded by a marker.

Sixty experiments were performed. No contraction was observed in 22 experiments using frequencies of 200, 500, 1,000, and 1,500 cps. On further increase of frequency to 2,000, 2,500, and 3,000 cps, the muscles showed a small contraction in all cases with no exception (17 experiments). Further, at 3,500 cps, contraction was obtained only in two experiments out of five, and at still higher frequencies — 4,000, 5,000, 7,000, and 10,000 cps, contraction was not observed in any one of 16 experiments. Thus, of the whole scale of sounds tested (200 to 10,000 cps), only a very narrow zone of frequencies (2,000 to 3,500 cps) caused muscle contraction (shaded region of Figure 11). The middle of this region coincides exactly with the maximum region of vital staining, while its right and left borders correspond to almost identical levels of increase in staining power (20.5-22.8%). It appears that denaturation changes of protoplasm proteins serve as a stimulus for those biochemical processes which cause muscle contraction.

Figure 12 shows several kymograms of contractions caused by sounds of different frequencies. The relatively long latent period between the onset of stimulation and the response reaction (from 1 to 5 minutes) is noteworthy. Contractions quickly reached a maximum, after which a slow relaxation of the muscle began. After 2-5 minutes, the muscle reverted to its initial length, although the action of the sound continued.

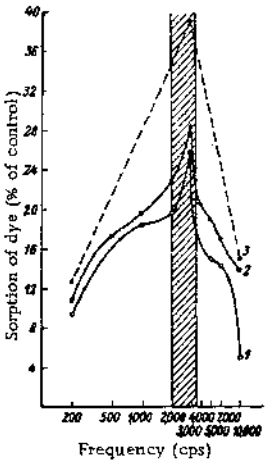


FIGURE 11. Enhancement of vital staining and appearance of contractions in the sartorius muscles of frog exposed to audible sounds of different frequencies at a constant level of intensity (94 db)

1, 2—neutral red (basic dye); 3—cyanol (acid dye). The shaded zone is the region of appearance of sonic contractions. The frequencies are given on a logarithmic scale (1—according to Ravdonik, 1949; 2, 3—according to Nasonov and Ravdonik, 1947).

The above-mentioned sonic contractions are easily obtained with spring-summer frogs (Nasonov and Ravdonik, 1947), considerably less easily with autumn-winter ones (Ravdonik, 1949), and with great difficulty with winter frogs, the muscle excitability of which is known to be lower than that of summer frogs. There is good reason to believe that in this case it is not a contraction but a rhythmic activity of individual fibers taking place. This is often observed at the very beginning of the stimulus, and is connected with the initial increase in excitability, only to be later replaced by a decrease in excitability. However, the nature of sonic contractions is a problem requiring further study.

37 In addition to experiments with sartorius muscle, a small series (16 experiments) was performed with the straight abdominal muscle of the frog. The experimental conditions were exactly the same as in previous cases. The results were very similar. Here, too, contractions appeared only within a narrow range of sounds of intermediate frequencies (2,500 to 3,500 cps). As in the experiments with the sartorius, there was a peculiar "resonance" of the muscles to a definitive frequency range.

Ravdonik (1949), by using precisely the same methods, further investigated the direct reaction of muscles to sounds, when only the intensity of the stimulus varied, and the frequency of vibrations was 3,000 cps. He studied the effect of sounds of the following intensities: 70-75, 80-85, 90-95, and 120 db. The results obtained are illustrated in Figure 13, which shows the threshold nature of the sound stimulus. For the frequency of 3,000 cps, a clearcut increase in staining power began at 90 db, while in the region of 96 db the level of the reaction approached a constant value. Sonic contractions appeared at 95 db (shaded region of the graph). Thus, here too, contractions begin simultaneously with paranecrotic changes in protoplasm.

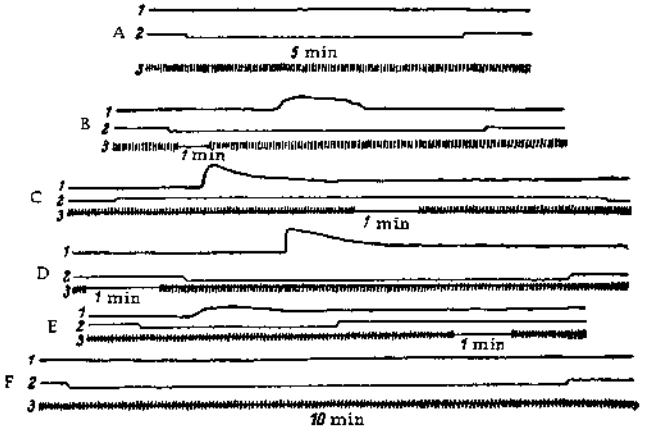


FIGURE 12. Myograms of sonic contractions (according to Nasonov and Ravdonik, 1947)

A—1,500 cps; B—2,000 cps; C—2,500 cps; D—3,000 cps; E—3,500 cps; F—4,000 cps. 1—muscle contraction; 2—duration of stimulus; 3—time marker.

Nasonov and Ravdonik (1950), using spinal ganglia nerve cells of rabbits and Romanov (1954), using sympathetic ganglia of rabbits, later investigated the action of sound on these organs by the staining technique. The ganglia of the lumbar region of the rabbit were isolated together with the nerve roots, from which the connective tissue sheath was removed. These
38 were stained for 20 minutes with 0.1 neutral red, prepared in Ringer's solution. The ganglia on one side were exposed to sounds of 120 db during staining, while the contralateral ganglia served as controls. After staining the ganglia, the nerve tips were cut off and the dye was extracted with acidified 70% alcohol. Subsequently, the extracts were measured colorimetrically, and the intensity of staining of the experimental ganglia was expressed as a percentage of the controls. The results are shown in Figure 14. It was shown that nerve cells, too, react to sound and, like muscle fibers, show a maximum area of reception in the region of 3,000 cps.

Finally, Nasonov and Rozental' (1950) discovered a similar reaction

to sound by cells of frog kidney epithelium* (Figure 15). Comparing the changes in muscles, nerve cells, and kidney epithelium (Figures 11, 14, and 15), it will be seen that in all these tissues the same maximum reaction occurred at 3,000 cps.

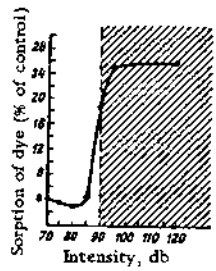


FIGURE 13. Increase in sorption of neutral red by sartorius frog muscles exposed to audible sounds, of various intensities, at a frequency of 3,000 cps. The shaded area is that of intensities causing contraction (according to Ravdonik, 1949).

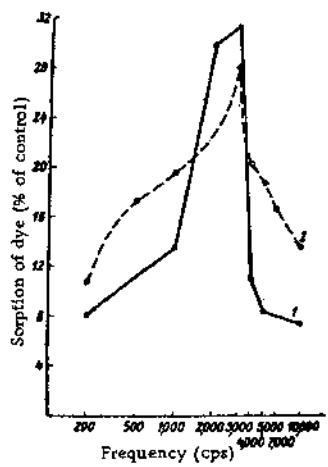


FIGURE 14. Staining power of neutral red of: 1—nerve cells of spinal ganglia of the rabbit; 2—frog muscles, under the influence of sounds of different frequencies at the same intensity (120 db). Frequencies are given on a logarithmic scale (1—according to Nasonov and Ravdonik, 1950; 2—according to Nasonov and Ravdonik, 1947).

---

* Kidney epithelium was stained only with an acidic dye (0.08% phenol red), since basic dyes cause marked granule formation in kidneys.

On this basis, it may be concluded that sounds in the region of acoustic
39 reception may act as direct physiological stimulants on cell protoplasm, without the participation of the auditory organ or the nervous system.

The question then arises: in what manner is sonic energy registered by the protoplasm?

Our hypothesis is based on the denaturation theory of damage and excitation. In our opinion, any agent causing denaturation of native proteins in vitro may serve as a stimulant, since protoplasmic protein (which can be easily denaturated) is the component which first receives the action of any stimulant. The capacity of sound to denaturate native proteins has already been established (Chambers and Flosdorf, 1936). The intimate mechanism of this process is as yet unknown. Its elucidation is a task for organic chemists.

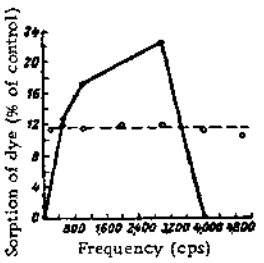


FIGURE 15. Changes in staining of frog kidneys with phenol red after excitation by sounds of different frequencies (according to Nasonov and Rozental', 1950).

The dotted line designates the intensity of stimulation used—120 db.

During the propagation of sound waves through any medium whatsoever, periodic increase and decrease in pressure take place. Rhythmic vibrations of the particles occur parallel to the direction of the wave motion, and there is some increase in temperature. It could be assumed that each of these changes may excite living protoplasm. But the changes in these measurements (in absolute values) are very small. For example, the amplitudes of oscillation of air particles were measured in micra; and on passing sound through liquid, as in our experiments on vital staining, or through protoplasm, the actual shift of the particles would need to be measured in A.

Mechanical (tactile) stimulation is feasible only if protoplasm is to some extent deformed, i.e., if displacement of the protoplasmic particles in relation to each other occurs. However, the length of the sound wave in liquid (about 0.5 meter at 3,000 cps) exceeds to such extent the dimensions of the muscle fiber that the relative displacements of the particles of the latter will still be many times less than the amplitude of the former. It is self-evident that such small deformations cannot cause a tactile stimulation of protoplasm.

Pressure increases only by millionths of fractions of an atmosphere upon propagation of sound of the strength used by us. It is quite improbable that such changes could influence protoplasm. It will be shown later that skeletal muscles of frog register hydrostatic pressure starting from 100 atmospheres, i.e., from values exceeding one hundred million times the pressure of sound of the strength used in our experiments.

40 The same is also true of temperature which, following the action of sound, increases by only 0.00001 degrees. But that cell protoplasm reacts to sound is beyond any doubt.

Small pressure changes may be magnified when the rhythm of the vibrating particles is in resonance with other structures. It may be assumed that it is the protein molecules in the cells, possibly denaturated by sounds of certain frequencies, which oscillate. In this respect, the maximal reaction of 3,000 cps on the staining power (curves 11, 14, and 15) and the corresponding zone of contractions are of special interest. These phenomena may be due to resonance. Another explanation, given by the physicist Frenkel (1948), was that of relaxation oscillation. Neither theory is fully confirmed.

It is interesting to note that sound waves which are most easily perceived by the human ear are similarly perceived by muscles. In Figure 16, the sensitivity curve of frog muscles to sound of different frequencies and the sensitivity curve of the human ear (threshold of sound perception in a free sonic field) are compared by the method of vital staining according to the data of Sivian and White (1933). The similarity of the two curves can hardly be explained by simple coincidence.

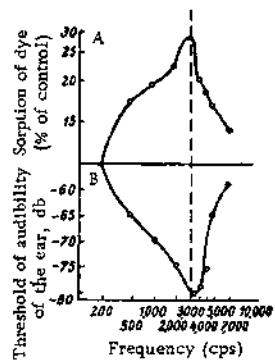


FIGURE 16. Action of sound of different frequencies on (A) sorption properties of frog muscles, (B) thresholds of audibility of the human ear (according to Nasonov and Radvonik, 1947). Sorption of the dye is given on a logarithmic scale. In the calculations, one bar was considered as zero db.

The changes in muscles occurring after optimal oscillations of 3,000 cps are probably characteristic of changes in cells of many other organisms.

It is highly likely, therefore, that the capacity of protoplasm to receive direct sound stimuli is accentuated by the development of the auditory apparatus, whose receptor cells may contain protoplasm with different

41 sensitivities to frequencies within the normal range of hearing*. In this case, the whole complicated structure of the organ of Corti and the transmitting mechanism of the middle ear could be considered as an additional apparatus, which appeared in the process of evolution, acting mainly as a potent sound amplifier, while the function of the peripheral analysis of tones is performed mainly by the auditory epithelial cells themselves, the protoplasmic proteins of which may be "tuned" to different tones.

This theory explains the mechanism of sound perception by animals which do not possess an organ of Corti, but are nevertheless able to distinguish tones (e.g. fishes). It also explains certain difficulties connected with the resonance theory of Helmholtz.

Reaction of Protoplasm to High Hydrostatic Pressure

This reaction is of special interest. Since high pressure does not affect the process, vital staining can be performed during the action of the stimulant. This stimulant has another advantage compared with other agents, in that it may be applied instantaneously, and be completely removed. Furthermore, it acts simultaneously on the whole object, including its very deepest parts. For us, it is of interest also because there are quite convincing data in favor of the contention that the reaction of living protoplasm to hydrostatic pressure is explained by the denaturating effect of this agent on native proteins.

In experiments of this kind, it is essential to remove gases from the system, since an increase in pressure may increase the solubility of gases. On decompression, gases in the form of bubbles may damage the protoplasm. In addition, even gases like nitrogen may acquire toxic properties under pressure (for further details, see Lazarev, 1941).

There are many papers on the influence of pressure, and there are comprehensive reviews of the literature up to 1948 (Cattell, 1936; Ebbecke, 1944; Tongur, 1947; Bridgeman, 1948).

The general conclusion which may be drawn from the latter is that pressure of a sufficient magnitude (of the order of several hundred atmospheres) causes inhibition and reversible suppression of various vital functions (e.g., cessation of motion of animals, contraction of striated muscles, cardiac arrest, contraction of heart muscle, suppression of the rhythmic activity and contraction of smooth muscles, decrease or arrest of nerve excitability, etc.). In a number of cases, at a lower pressure a stage of functional activation may be observed (increased motor activity, an acceleration and increased amplitude of heart muscle contraction and of that of smooth muscles; a lowering of the nerve threshold response, and multiple reactions to a single stimulus of the nerve).

42 The action of pressure on striated muscles was studied in great detail (Ebbecke, 1914, 1923, 1935a, 1935b, 1936, 1935; Fontaine, 1928a, 1928b, 1929; Cattell a. Edwards, 1928, 1932; Edwards a. Cattell, 1932; Brown a. Edwards, 1932; Brown, 1934, 1935, 1936a, 1936b; Ebbecke u. Hasenbring, 1935, 1936; Ebbecke u. Schaefer, 1935; Hasenbring, 1940).

---

* There is a strong basis for the assumption that the thresholds of perception of sonic stimuli by the protoplasm are considerably lower than the thresholds of action of sounds on protein in vitro.

It was found that the first flicker of contraction of frog muscle may sometimes be observed at 150-180 atmospheres, but in general contraction took place on increase of pressure to 200-300 atmospheres. The muscle response to stimulation by pressure is of an obviously gradual nature: higher pressure caused greater contraction of the muscle (Figures 17 and 18). Such contraction was freely reversible: on decompression the muscle returned to its initial length, and only at pressures above 450 atmospheres or upon prolonged action of lower pressures, did the contraction remain after the pressure ceased (Figure 18, pressure of 500 atmospheres).

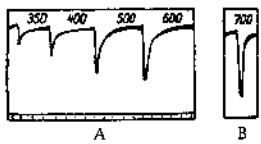


FIGURE 17. Contraction of muscle after pressure of very short duration ("pressure shock") (according to Ebbecke and Hasenbring, 1935)

A—pressure of 350, 400, 500 and 600 atmospheres; B—pressure of 700 atmospheres; time in seconds.

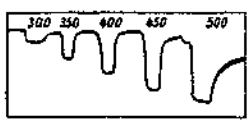


FIGURE 18. Contraction of muscle after pressure of different strengths and a duration of one second (according to Ebbecke and Hasenbring, 1935). The numbers indicate pressure, in atmospheres

Ebbecke draws an analogy with contractions caused by different chemical and physical stimuli, and he uses the term "mechanonarcosis" in the case of pressure. In his opinion pressure as a stimulus is closest in its properties to electric stimulation, and contraction due to pressure is very similar to muscle contraction under the cathode (the so-called "cathodic barrage"). The only difference is that in the latter case local contraction takes place, while pressure simultaneously influences the whole muscle. Ebbecke also points out that contraction by pressure is fully analogous in its height and course to a single contraction and a short tetanus. This analogy is also confirmed by the fact that, as will be seen later, the same biochemical processes take place as with a normal muscle contraction caused by a stimulus conducted by the nerve.

In our laboratory, the reaction of muscle to changes in hydrostatic pressure was investigated by Golovina (1955a). In order to determine the degree of paranecrosis, the quantitative method of vital staining was employed.

In order to create high hydrostatic pressure, a hydraulic press and special reactors were used, i.e. thick-walled steel cylinders with a narrow
43 chamber in the middle. The chamber was filled with liquid, and the aperture was closed with a steel punch. A rubber (tightening) gasket was used.

A sartorius frog muscle was placed inside the chamber, in a glass test tube filled to the brim with the dye solution and stoppered with a rubber stopper, through which pressure was transmitted to the tube. In all the experiments, special attention was paid to avoid air bubbles.

The staining, extraction of the dye and comparison of sorption by the experimental and control muscles were performed as described in the experiments with sound. The action of pressure with simultaneous staining lasted 30 minutes. Neutral red and phenol red dyes were used in dilutions of 0.01%, prepared in Ringer's solution.

Firstly it was shown that increase in hydrostatic pressure had no effect on the staining of dead tissues. Muscles previously killed by alcohol did not show differences in sorption staining under a pressure of 1,000 atmospheres, as compared with controls stained under normal pressure. However, in living muscles a difference was invariably seen in staining when pressurized and nonpressurized muscles were compared.

Table 9 and Figure 20 give data on staining of muscles with neutral red. In the course of experiments, a difference was observed in the reactions of muscles of winter and spring frogs, the muscles of the latter being more sensitive to pressure. At 100 atmospheres, they already showed a decrease in sorption of 15.3%, while the sorption properties of the former
44 were not affected. Only at a pressure of 200 atmospheres was a decrease in staining of muscles from winter frogs seen (-22.2%).

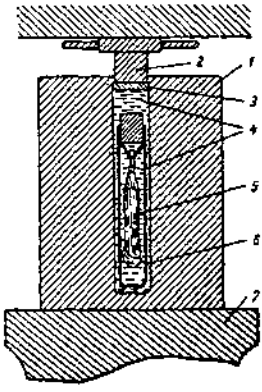


FIGURE 19. Drawing of high-pressure chamber (according to Golovina, 1955a)

1—steel cylinder; 2—punch; 3—gasket; 4—dye solution; 5—muscle; 6—test tube; 7—press surface.

Pressures of 100 and 200 atmospheres were below the threshold. However, investigations of the effect of pressure which causes contraction show a different picture. At a pressure of 400 atmospheres, the pressurized muscle stained more strongly than the control. The significant difference in staining was + 39.2%. This difference was even greater with increase in pressure. At a pressure of 1,000 atmospheres, it reached + 60.4%.

Thus, the relationship of dye sorption to the magnitude of pressure is biphasic; decreased sorption at a "subthreshold" value of pressure is replaced by increased sorption at a higher pressure (Figure 20).

In order to elucidate whether the changes obtained in sorption were not a result of shift in pH under the influence of pressure or granule formation, Golovina performed experiments with the diffusable acid dye—phenol red.

45 The results of these experiments are given in Table 9 and in Figure 21. At 100 atmospheres a decrease in sorption of 9.7% was observed; at 400 atmospheres—an increase of 36%; and at 1,000 atmospheres—an increase of 76.9%.

Thus, a biphasic curve was also observed upon staining with a diffusible acid dye. This proves that neither pH changes nor the influence of granule formation cause the results obtained. Sorption of the dyes by muscles protoplasm under the influence of high pressure is a genuine phenomenon.

When muscles were removed from the chamber during the phase of lowered absorption, in the majority of cases they did not differ from the controls, although sometimes they were somewhat shorter. Probably a 200-atmosphere for 30 minutes was for certain muscles already a threshold limit. Contraction probably occurred at the end of the stimulus, since

a 10-minute pressure did not cause contraction. Muscle excitability, as measured by the discharges of a condensor of 30 microfarads immediately after decompression, was somewhat increased, and continued to rise for several minutes. In a number of cases, the muscle contracted for 3-12 minutes. After 10-15 minutes, excitability began to decrease, sometimes down to the initial level, or below it. The results of an experiment of this type are illustrated in Figure 22.

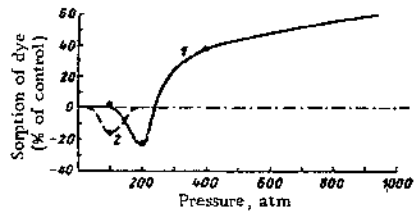


FIGURE 20. Binding of neutral red by sartorius frog muscle during the action of pressure (according to Golovina, 1955a)

1—winter frogs; 2—spring frogs.

Table 9

Changes in sorption properties of sartorius frog muscle during the action of increased hydrostatic pressure (staining as a percent of dye control) (according to Golovina, 1955a)

Dye	Season	Pressure, atm.			
		100	200	400	1,000
Neutral red	Winter	+ 2.5 ± 5.2	- 22.2 ± 3.9	+ 39.2 ± 9.6	+ 60.4 ± 7.1
Neutral red	Spring	-15.3 ± 3.4	—	—	—
Phenol red	Spring	- 9.7 ± 2.0	+ 3.6 ± 12.7	+ 36.0 ± 9.8	+ 76.9 ± 13.0

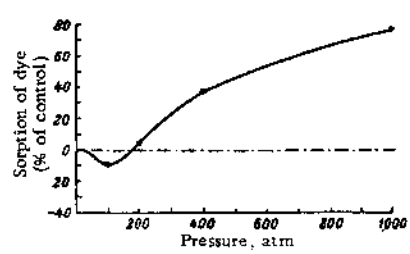


FIGURE 21. Binding of phenol red by the sartorius muscle of spring frog during the action of pressure (according to Golovina, 1955a)

A 5-minute staining of the muscle, one minute after decompression (in the period of the greatest increase in excitability) led to decrease in sorption of 17.8%. This condition of lowered staining power probably disappeared as quickly as the condition of increased excitability, since a

46 20-minute staining gave a considerably smaller and statistically insignificant result: ± 8.3 ± 4.4% (Golovina, 1958a).

Golovina (1958b) also investigated the brains of white mice.

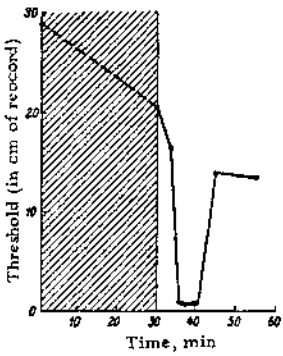


FIGURE 22. Influence of 200-atmosphere pressure on excitability thresholds of the sartorius muscle of the frog (according to Golovina, 1958a)

The rhythmic contractions of the muscle are shown by diagonal shading. The shaded area designates the duration of pressure action on the experimental muscle.

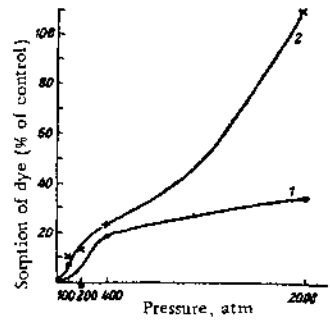


FIGURE 23. Change of sorption properties of the brain of a white mouse under the influence of high hydrostatic pressure (according to Golovina, 1958b). 1--sorption of neutral red; 2--sorption of phenol red.

Such a brain, from which the membranes were removed, was stained while under pressure for 10 minutes at 17-20° C. Another brain from an animal of the same batch served as a control. The dyes used were neutral red (0.1% solution) and phenol red (saturated solution). The results are illustrated in Figure 23, from which it will be seen that the basic dye (neutral red) caused a clearcut increase in staining, starting from a pressure of 400 atmospheres. Staining with phenol red shows a greater increase in sorption at all points, which at 400 atmospheres reached 22%, and at 2,000 atmospheres 108%. Thus the action of high pressure on the brain clearly increased the sorption of both acid and basic dyes, and, as in the case of the muscles, increase was not due to any change in pH.

On comparing the reaction of the brain with that of the muscles, no stage of decreased sorption is detected in the former, while it is well marked in the latter. It is quite possible that this is associated with the worse condition of the "surviving" brain as compared with the surviving muscles.

Finally, Suzdal'skaya (1955) studied in our laboratory the effect of high pressure on the in vivo binding of basic and acid dyes by the pancreas and salivary glands of the mouse. Her data are illustrated in Figures 24, 25, and 26. These show that the cells of salivary epithelium were much less sensitive to pressure than muscle and nerve tissue cells. Only a pressure of 1,000 atmospheres caused an increase in staining, greater with acid than with basic dye. Subthreshold pressure applied to glands and muscles led to a small decrease in staining of salivary epithelium.

The action of pressure on a living system has not been fully explained, and various authors have approached it from different points of view.

Thus, Cattel (1936), ascribed a greater importance to colloid changes in protoplasm, and hence to change in viscosity and in viscous-elastic properties. Ebbecke (1937, 1944), as already indicated, held that pressure acts
47 as any narcotic does, and proposed the term "mechanonarcosis".

Johnson, Marsland, and other authors (Brown, Johnson and Marsland, 1941; Johnson, Brown and Marsland, 1942a, 1942b; Johnson, Eyring, and Williams, 1942; Johnson and Eyring, 1948; Bronk, Harvey and Johnson, 1952) concluded that the effect of pressure on the intensity of bacterial luminescence is explained by its effect on the protein structure of the enzyme, luciferase. Under various conditions of equilibrium between the denaturated and native forms of luciferase, the equilibrium shifted in certain cases towards a denaturated form, with decrease in luminescence, or, alternatively, in the direction of the native form, leading to increased luminescence. In the opinion of these authors, the essence of pressure action is its interference with denaturation occurring with increase in volume, and in a shift in equilibrium in the direction of a greater amount of the native form of luciferase.

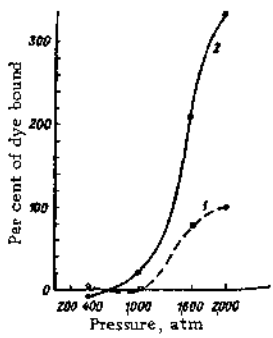


FIGURE 24. Binding of dyes by the pancreas, depending on the level of hydrostatic pressure (according to Suzdal'skaya, 1955)

1—neutral red; 2—phenol red.

Although not all the points of the above authors' concepts have been confirmed, the presentation of the problem is highly interesting and promising.

The data obtained in our laboratory by Golovina (1955a, 1958a, 1958b) and by Suzdal'skaya (1955) on increased sorption properties of protoplasm due to high pressure also prove that cell proteins under these conditions undergo changes similar to denaturation. A further proof is the observation of Deuticke and Ebbecke (1937) on the decrease in solubility of muscle proteins following pressure.

There is also direct evidence of the susceptibility of proteins to pressure in vitro. Bridgman (1914), using egg albumin, observed a considerable increase in density at 5,000 atmospheres, and complete coagulation at 7,000 atmospheres. Later, curdling and coagulation were observed by Basset, Macheboeuf and Sandor (1933); Dow et al. (Dow and Matthews, 1939); Laufler and Dow, 1941); and finally by Tongur (1948, 1949).

Many authors have described the inhibitory effect of pressure on protein denaturation by different agents (Johnson and Campbell, 1945, 1946; Johnson and Wright, 1946).

48 Tongur and co-workers studied the restoration of proteins by high hydrostatic pressure (Tongur, 1947, 1948; Tongur and Kasatochkin, 1950; 1952; Tongur and Kazmina, 1950; Tongur and Tongur, 1951). The authors observed a return of denaturated protein to its native condition under the influence of pressure. Pressures causing such restoration are lower than those capable of causing denaturation of proteins (the latter starting from 700 atmospheres).

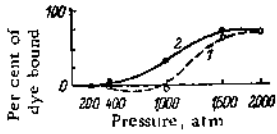


FIGURE 25. Binding of dyes by the submaxillary gland, depending on the level of hydrostatic pressure (according to Suzdal'skaya, 1955)

Legend as in Figure 24.

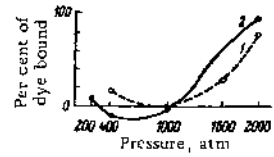


FIGURE 26. Binding of dyes by the parotid gland, depending on the level of hydrostatic pressure (according to Suzdal'skaya, 1955)

Legend as in Figure 24.

Thus, a sufficiently high hydrostatic pressure is capable of denaturating native proteins in vitro, while low pressure may restore them. These data aid greatly in elucidating the results obtained by the method of vital staining.

Chapter 3. Local Reaction and Stimulation

Excitation and Damage

To date, there is no generally accepted and established definition in physiology of the term "excitation"*. Therefore every physiologist, in using the term, should clearly define his interpretation of it.

The present book deals only with cellular excitation and not with stimulation of the whole organism, which means something quite different. "Cellular excitation" implies reversible changes in protoplasm occurring under the influence of changing external conditions.

On the basis of a series of data, it is assumed that excitation of protoplasm is characterized by specific physiochemical and biochemical properties, and that the phenomenon has developed in the process of evolution, as a condition essential for the existence of any living organism.

This definition of the term "excitation" corresponds to the highest known form of excitation of protoplasm resulting from an impulse travelling along a nerve or muscle fiber.

The same may also be said of excitation of secretory cells, various receptor elements in sensory organs, mature egg cells after fertilization by spermatozoa (or parthenogenesis in cases of artificial parthenogenesis), connective tissue and epithelial cells which, due to stimulation, pass from the inactive to an active state, or proliferate, etc. It should be pointed out that the last of these examples has been little studied as yet. Thus, data on excitation is obtained mainly from the study of nerve or muscle tissues.

In this connection arises the problem of how to consider the local cell reactions due to alterations of protein molecules (similar in nature to the
49 initial stages of denaturation of native proteins in vitro) expressed by paranecrotic changes in the protoplasm.

---

* A review of the literature and attempts to analyze the term "excitation" may be found in the papers by Mangold (1925) and Frédéricq (1928).

We treated these changes in protoplasm mainly as "reversible damage", a term which met with no opposition (Nasonov and Aleksandrov, 1940). Objections appeared only when the problem at hand was defined as "the reaction of living substance to external influences". We were told, and at first sight with good reason, that simple damage of alteration is not yet a "reaction", since by its meaning this word (reaction—reverse action) should designate a process caused by the action of the agent and directed against it. If such a process does not take place, but there is only a passive change of the system under the influence of external force, we are justified in speaking only of "damage" or "alteration". In discussing paranecrotic changes in protoplasm, only reversible change, repairable by the cell itself, is implied. It is assumed that this repair may be achieved only by active participation of cell metabolism. Consequently, paranecrosis is not only a passive "denaturation" of protoplasm proteins, but also a mechanism initiating a chain of metabolic reactions directed to its repair. This being so, paranecrosis may not only be termed "reaction", but also by our definition "excitation".

Unfortunately, there are no concrete data from which the properties characteristic of the primordial primitive organisms can be deduced. Primitive organisms, being only fragments of protoplasm, should be able to repair such damage to protein structure which results from drastic environmental changes. Without this, the existence of these organisms would not be feasible. This property of cell repair is probably as basic and as old as that of metabolism, cell division, and growth. From this point of view the reversible damages of protoplasm may be regarded as the most primitive form of excitation. This being so, paranecrosis is not only the simplest and most common, but phylogenetically the most ancient, form of excitation.

If the first step in the evolution of excitation was the ability to repair damage which itself was a stimulus for repair, the next step should have been protection. Damage itself should have become a release mechanism, not only of biochemical processes leading to repair, but also of such processes which conditioned either defense against danger or its avoidance (e.g. withdrawal of pseudopodia, movement of the organism away from the harmful agent, i.e., various tropisms*, or by secretion of protecting substances, etc.).

50 Higher in the evolutionary scale, with development of the nervous system, the most perfect form of excitation appeared—the spreading excitation. This enabled transmission of signals from the part of the organism subjected to environmental change (the receptor), to another part of the organism which was the effector mechanism. This spreading excitation not only modified the behavior of the organism in relation to the surrounding environment, but also coordinated the activity of different parts of the organism with respect to each other.

---

* This theory explains only negative taxis and tropism. The positive ones, manifested not in avoiding harm but in striving toward something expedient, require another explanation. However, taxis and tropisms are always expressed in motion or growth along a certain gradient, and therefore withdrawal from harm is also a striving towards the expedient. Thus, if an organism moves away from low temperature, this is equivalent to an attempt to reach a higher temperature. Later, in the course of evolution from these primitive forms, more specialized types of taxis developed, such as chemotaxis.

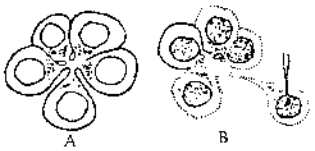


FIGURE 27. Irradiation of a local reaction through protoplasmic bridges in spermatogonia of a grasshopper pricked with a microneedle (according to Chambers, 1925)

A—rosette of 5 interconnected spermatogonia; B—coagulation of nuclei after pricking one of them with a microneedle.

In our 1940 monograph, there was much evidence that local reaction is capable of spreading not only in the nerve elements, albeit slowly and with gradual fading. As an example we mentioned the old observation of Verworn (1896a, 1896b) on irradiation of damage caused by prickling the pseudopodium of a rhizopod (Hyalopus) with a needle. This damage was reversible, manifested as a spreading turbidity of the protoplasm and contraction and withdrawal of the pseudopodia. Another example was taken from a paper by Chambers and Renyi (1925). These investigators pricked living cells of the multilayered squamous epithelium of mammals with a micromanipulator needle. These cells are interconnected by protoplasmic bridges. Soon after pricking one of the cells, signs of paranecrosis appeared in its protoplasm and nucleus. After some time, similar changes appeared in neighboring nonpricked cells and later paranecrotic changes began to irradiate from the damaged area along the intracellular bridges to involve ever-increasing sections of tissue. In epithelia without intracellular bridges, no irradiation of damage was observed. Such an irradiation of local reaction was observed by Chambers (1925) upon pricking one of the spermatogonia of a grasshopper, connected with others by protoplasmic appendages. Nuclear changes were first seen in the pricked cell and later in all the others connected with it by plasmodesmata (Figure 27). Later, Chakhotin (1935) described irradiation of damage caused by radial pricking of a segment of erythrocyte protoplasm.

Finally, a good example of the spread of local protoplasmic reaction may be observed on local damage of a striated muscle fiber. Cutting of, or pressure applied to, such a fiber, produced a swelling in the damaged zone. 51 The latter strongly refracts light, and stains intensely with vital stains. However, the borders of this damage never remain fixed. They soon begin to move along the fiber, involving large sectors of living protoplasm, as if the damaged protoplasm caused the death of neighboring intact regions. In our laboratory this phenomenon was studied in detail by Raevskaya (1948), using the method of "time-stop" photomicrography. Among other things, she showed that in the absence of calcium ions, the damage in the muscles practically did not spread. Further study showed that spread of damage along a healthy fiber is due to an electric mechanism (Rozental', 1946, Nasonov and Rozental', 1947).

The basis for this assumption was an observation that the velocity of spread of the damage is very small, i.e. about 0.04 mm per hour, in a muscle located in a medium of low electroconductivity (for example, in a moist chamber), while in media which are good conductors of electric current (e.g. Ringer's solution), this process is much faster (about 0.3 mm per hour). It was suggested that the reason for change in the protoplasmic sector adjacent to the damaged one was a resting current, which was closed through the surrounding medium, as shown in Figure 28. In other words, it was assumed that the mechanism of damage irradiation was similar in nature to the mechanism of irradiation stimulus in conducting fibers.

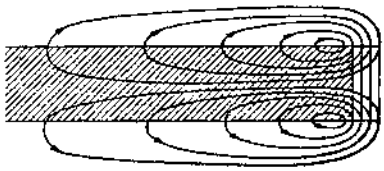


FIGURE 28. Schematic description of "small currents" of the muscle fiber (according to German) in the region of the cut (according to Nasonov and Rozental', 1947)

In order to confirm the above assumption it was necessary to create an environment in which two muscles were kept under exactly the same life conditions, but for one of them, the resistance to closure of the resting current through the surrounding medium was greater than for the other. For this purpose one of the muscles was placed on an agar-agar layer 2 mm thick, diluted with Ringer's solution, while the other was placed on a layer of agar approximately 0.08 mm thick. The results of the experiments (see Figure 29) completely confirm the theory of the electric mechanism of irradiation. The spread of damage was faster on the thick agar layer, slower on the thin one, and hardly observed at all on glass.

Later, S.N. Aleksandrov investigated the velocity of spread of damage in muscles in relation to the fiber diameter (1948a), and temperature (1949). He also studied the special nature of spread of damage in tonic muscles (1955). It is well known that the velocity of spread of a stimulus along nerve fibers is directly proportional to their diameter. The same relationship
52 was found by Aleksandrov for the velocity of spread of damage in muscles. Thus there is another feature common to spread of damage and to excitation.

The same conclusions on the mechanism of irradiation in muscles were reached independently by Rotschuh (1955), in a paper that appeared considerably later than ours, and this author, too, correlated the phenomenon with spreading of physiological excitation in nerve fibers*

It is quite possible that the ability to transmit local excitation rapidly along nerve fibers over a long distance evolved from those primitive phenomena of irradiation of local reactions of protoplasm, which may even now be observed in various cellular elements not specialized to transmit impulses.

All these observations form a basis for the theory that reversible change in protoplasm occurring as a reaction to changes in environment is the most ancient form of excitation. On the basis of this excitation there evolved the perfect form of neural transmission for the whole animal kingdom. This does not imply that on passage of a stimulation wave along the nerve fiber, the same processes occur in the protoplasm of the latter as following the action of any stimulant on an epithelial or connective tissue cell. Undoubtedly, in the process of evolution, the denaturation changes of protoplasm proteins seen in ordinary paranecrosis might have acquired a special character adapted to rapid appearance during short-lived electric stimulus, and similar rapid repair. However, it is also beyond doubt that such apparently different phenomena as nervous and muscular stimulation on the one hand and reversible damage, on the other, have many common features. This is especially well seen in the so-called muscular contractions which will be presently discussed.

---

* Rotschuh, who was evidently unaware of our work, does not cite it, and claims that he was the first to propose the theory of electric mechanism of damage spread.

Excitation, Damage and Narcosis of Skeletal Muscles

The idea of a relationship between protoplasm damage and protoplasm excitation is very old, and it is difficult to establish which physiologist first formulated it. It is based on the obvious fact that all the agents causing
53 excitation may also cause protoplasm damage if they exceed a certain level. Also, many manifestations of excitation, such as electronegativity, release and exit from the protoplasm of certain electrolytes and a decrease in excitability (refractivity), are characteristic of damaged protoplasm.

On the basis of in vivo studies of the cytoplasm and nucleus of different cells, we frequently correlated these two conditions (Nasonov and Aleksandrov, 1940; Nasonov and Rozental', 1947; Nasonov and Ravdonik, 1947; Kiro, 1948). Later, in a series of special experiments on muscles, we tried to determine the sequence of appearance of manifestations of excitation and damage on increasing the strength of the stimulus.

In the majority of these studies the sartorius frog muscle was used, because of its homogeneous structure. In certain cases only, the straight abdominal muscle was used. Different stimulants were used, and the contractions studied.

The natural contraction of skeletal muscles is the so-called tetanus. As in the case of nerve fibers, an electric mechanism is the means by which the stimulation wave travels along the muscle. (For further details, see Part IV of this book). Pure contractions, i.e., contractions of long duration, not tetanic, but of a stable, nonoscillating nature, are not seen under natural conditions. As shown by Zhukov and his co-workers, prolonged tonic contractions, as observed in different pathological conditions, are something between natural tetanus and a stable nonfluctuating contraction, as in healing fractures of extremities, or in certain special cases (tonus of the extremities of the frog in the mating period, constrictor muscles of the mollusk shell, etc.) (Zhukov, 1956).

Nevertheless, the study of contractions is of great interest to the physiologist since natural tetanus may be considered as a series of consecutive contractions, caused by a cathode current.

Most of the literature available on muscular contractions was collected and analyzed in the beautifully compiled review by Gasser (1930), after which no fundamentally new research has been done in this field*.

Following, to some extent, his arbitrary classification, muscular contractions are divided into three groups: 1) veratrine-like contractions; 2) contractions of the acetylcholine type occurring under the influence of agents specifically acting on the receptors; 3) nonspecific contractions appearing in any muscle under the influence of various physical and chemical stimuli.

54 We only investigated the latter group in relation to varied agents used in a sufficiently large dose, e.g. temperature, radiant energy, electric current, hydrostatic pressure, mechanical action, audible sounds, acids, alkalis, salts, narcotics, alkaloids, etc. In this case, the contraction may be considered as the most typical, nonspecific response reaction of the living cell to any change in the external environment.

---

* For more recent literature, see Cuffler, 1946.

Gasser reached the same conclusion, on the basis of extensive literature. In his opinion, contractions possess all the attributes of stimulation of muscle tissue. Indeed, contractions are accompanied by tension with which mechanical work, characteristic of the muscles, may be performed. Contractions are accompanied by heat formation. The area of muscular contraction is electronegative in relation to the resting part. Finally, in contractions, all the elements of metabolism of muscle at work were found (see below). A basic difference between the contractile and tetanic shortening of muscles is the fact that the latter are not due to continuous waves of stimulation, but to stable, nonoscillating stimulation. The magnitude of this, as expressed by the height or force of the contraction, is directly proportional to the strength of the stimulus. In other words, it is gradual and the "all or none" law does not apply to it (Figure 30).

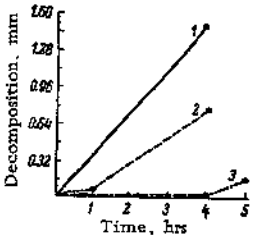


FIGURE 29. Spreading of damage in muscle fibers, depending on electric conductivity of the medium (according to Nasonov and Rozental', 1947)

1—on an agar layer, 2 mm thick; 2—the same, 0.08 mm thick; 3—on glass.

As will be seen later, the majority of stimuli possess their own threshold of action. On gradually increasing the strength of the stimulus, a narrow zone may always be found, where the response reaction first appears in the form of weak contractions. With the determination of these thresholds, the lowest intensity of the given agent sufficient to act as a physiological stimulus can be established.

We also tried to establish at what intensity this agent acts as a damaging factor. For the determination of this threshold, and for objective evaluation of the degree or depth of protoplasmic damage in muscle fibers, the above-described colorimetric method was used.

It has already been mentioned that for many years we studied these nonspecific changes in living protoplasm of various cells following the action of any stimulant (Nasonov and Aleksandrov, 1940). We concluded that these changes are always similar, regardless of whether they are caused by a physical or a chemical agent. A detailed investigation led us to believe that these changes depend on alterations in protoplasmic proteins in vitro, termed paranecrosis. This was also confirmed by Makarov (1938), who, using different materials, from protozoa to muscle and nerve cells of vertebrates, found that paranecrotic changes occur almost simultaneously with cell narcosis, which is a sort of functional expression of protoplasm damage*.

Thus there are two quantitative methods of evaluation of cell damage. Damage of living substrate was measured colorimetrically (curves B in Figures 33-35). The functional damage was measured by the appearance of cell narcosis (complete loss of electric excitability of muscles—curves A in Figures 33-35). Both these values are in direct, quantitative dependence on the strength of the stimulus.

---

* Here and later the term "cell narcosis" will be used in the widest sense, designating reversible loss of excitability caused by any agent whatsoever.

We attempted a comparison between the intensity of the stimuli causing local stimulation (contraction) with the intensities causing damage (narcosis, paranecrosis). When this work began we anticipated obtaining results according to the Arndt-Schulz rule (i.e. an increased dose of the agent first acts as a physiological stimulant, and later as a damaging factor, causing suppression of function, narcosis, and death). However, our conclusions were different and applied to any of the chosen stimulants.

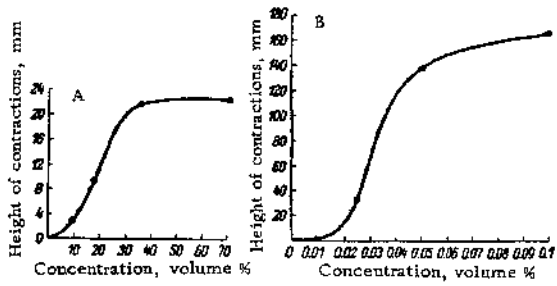


FIGURE 30. Relationship between the force of muscular contractions and the concentration of the substance used

A—ethyl alcohol; B—quinine hydrochloride; (A—from Nasonov and Suzdal'skaya, 1948; B—from Nasonov and Rozental', 1948).

In order to study the narcotic action of chemical agents causing muscle contractions, a series of dilutions of the substances in Ringer's solution were used. Each solution was twice as strong as that preceding it. The narcotic action of each concentration was determined by the time of onset of complete cell narcosis, i.e., a condition of the muscle when it did not respond to direct stimulation by induction current even by localized, hardly noticeable contractions. The current was supplied by the coils of a DuBois-Reymond sledge apparatus, completely drawn together and fed by a 4-volt battery. When cell narcosis occurred, the time of immersion in the solution under test was measured. The muscle was immediately transferred to pure Ringer's solution in order to determine the reversibility of narcosis. The results were illustrated in the form of logarithmic graphs (Figures 31-35).

56 It has been shown that the time curves characteristic of the narcotic capacity of a substance can be divided into two categories.

The curves of the first category are illustrated in Figure 31. The line ab corresponds to the duration of time which frog muscles survived in Ringer's solution. At room temperature, this was 2-3 days (3,000-4,000 minutes). Nasonov (1949a) and Ushakov et al. (Il'inskaya and Ushakov, 1952; Ushakov, 1953a, 1953b; Lopatina, Ushakov and Shapiro, 1953; Ushakov and Dzhamusova, 1954; Ushakov and Krolenko, 1954) have shown that progressive increase in the concentration of these substances, starting

from a very low value, shortens the survival time of frog muscles very gradually. A straight line is obtained, having a comparatively acute angle (α) to the horizontal (Figure 31). This means that with increased con-
57 centration of the substance (C) in Ringer's solution, the time of onset of complete narcosis (t) is always shortened in compliance with the same law which may be expressed by the empirical formula $t = \frac{\alpha}{C_n}$ (at sufficiently high values of C*).

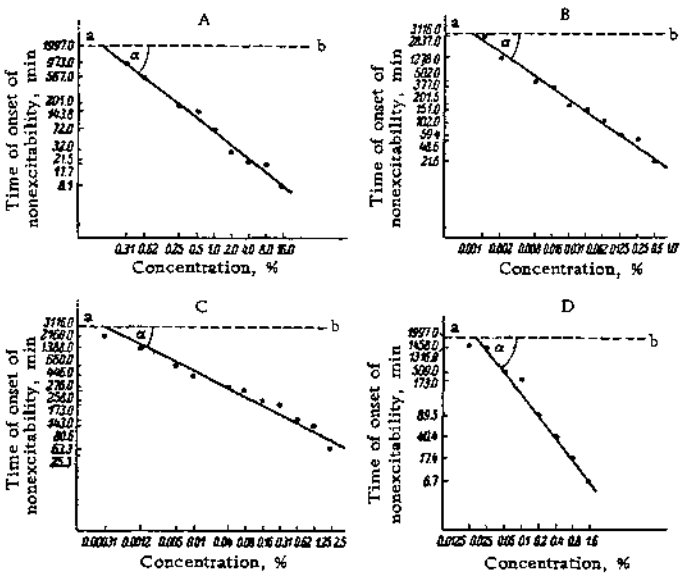


FIGURE 31. Nonthreshold relationship between the concentration of the substance tested, and the onset of muscular nonexcitability (narcosis)

A—cadmium sulfate; B—malachite green; C—monoiodoacetic acid; D—sodium fluoride. (A, B, D—according to Ushakov, 1953a, b; C—according to Ushakov and Krolenko, 1954). For further details, see text.

Among this group, according to Ushakov, are metabolic inhibitors, thiol poisons, and temperature. Lozin-Lozinskii (1955), also includes substances causing a photodynamic effect. For the majority of substances, another, more complicated rule exists, illustrated in Figures 32–35. This will be discussed, taking the effect of $MgCl_2$ on frog muscles as an example.

---

* The logarithm of the formula $t = \frac{\alpha}{C_n}$ is: $\log t = \log\alpha - n\log C$. This is an equation of the straight line, where n determines the slope of the line with the abscissa axis, and $\log\alpha$ gives the position of the line in the coordinate system.

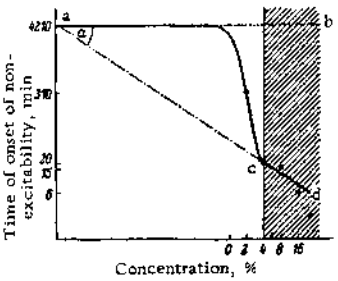


FIGURE 32. Threshold relationship between the concentration of $MgCl_2$ and the onset of muscular narcosis (according to Rozental', 1948).

The shaded area represents concentrations causing contractions. For further details, see text.

Addition of this substance to Ringer's solution at first had no effect on the muscle. Increasing the concentration above 1% caused a rapid decrease in the time necessary to produce complete narcosis (Figure 32). The curve drops at almost a right angle and breaks at point c, after which further shortening of the time of narcotization is slowed down. At the point of the break (4% $MgCl_2$), complete narcosis occurred after 20 minutes, while at a 1% concentration the muscle survived, as did the control, for about three days. In other words, with a gradual increase in concentration of $MgCl_2$, its narcotic effect increased suddenly, and not, as in the substances illustrated in Figure 31, gradually. The curve relating the time of onset of narcosis to concentration was of the same nature for all the 13 chemical agents studied (Figures 33-35). The lower point of its inflection (c in Figure 32) at which narcosis of the muscle first started is of special interest. We shall call this point the threshold concentration, and the narcotic action of this type we shall call the threshold action, as distinct from the nonthreshold, gradual one illustrated in Figure 31.

Ushakov, studying these two types of cell narcosis, stressed the fact that the nonthreshold type is always observed when the chemical agent concerned is a metabolic inhibitor, paralyzing some link of intracellular metabolism. His theory was that in the case of threshold action, metabolic reactions directed towards repair of damage caused by the poison started
58 immediately when the narcotic entered the protoplasm, these reactions being first of all directed towards repair of denaturation changes in native proteins. As long as the concentrations of the poisons were not too high, the cell metabolism could cope with them. The cell could adapt to the action of the poison, and the muscle fibers did not lose their excitability in its presence. When the concentration of the poison reached a certain critical level, adaptation of the cell became impossible; metabolism could not "correct" the protein damage caused by the poison, and the curve of time of onset of narcosis dropped steeply. Nonexcitability of the muscle fiber began to develop quickly, at first being reversible and later irreversible.
59 Ushakov nicely explained the threshold nature of the narcotic action, whereby this value ought to be determined by the limit of adaptation of protoplasm to the poison*.

---

* Ushakov (Lopatina, Ushakov, Shapiro, 1953) put forward the theory that at concentrations above the limit of adaptation (shaded area in Figure 32), the velocity of onset of nonexcitability is determined only by denaturation of protoplasm proteins. The relationship between this and the poison content of the solution is given by the sector cd in Figure 32. According to Ushakov, if metabolism ceases completely, the threshold curve should transform into the straight line acd, and thus become similar to the other nonthreshold curves in Figure 31.

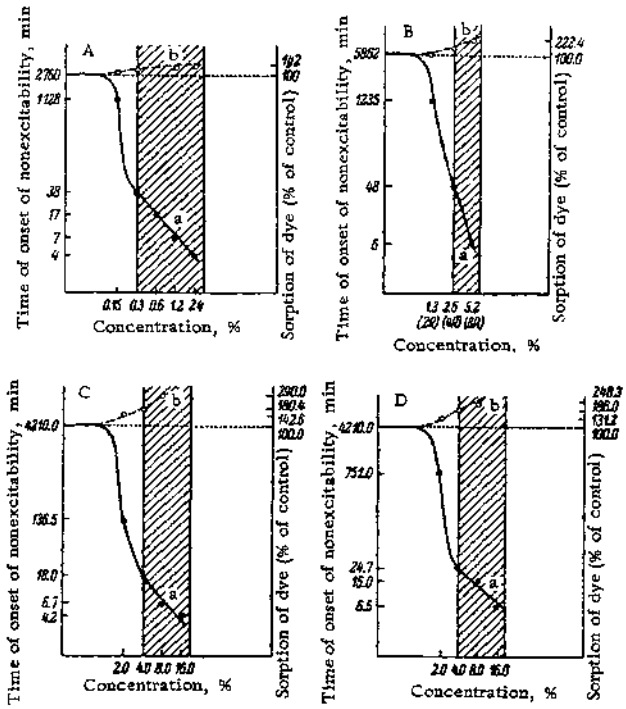


FIGURE 33. Relationship between concentration of the substance and (a) onset of nonexcitability; (b) change in staining power of frog muscles

A–KCl; B–NaCl; C–$CaCl_2$; D–$BaCl_2$.
The shaded area represents concentrations causing contractions (A, B–according to Nasonov and Suzdal'skaya, 1948, C,D–according to Rozental', 1948)

If, however, the poison acts as a specific inhibitor, suppressing metabolism already at low concentrations, repair of protoplasm damage does not take place, and a gradual increase in damage is observed with the increase in the dose of poison (Figure 31).

60 Since our aim was to compare cell damage and the onset of excitation, it was much more convenient to use substances of threshold action for this purpose than substances showing gradual action. With the threshold mechanism, the differentiation between the concentrations where no damages occur and those at which they do appear can be seen with certainty.

The effect of certain threshold substances will be discussed in greater detail. Table 10 and Figure 35 (A) show the concentration of ethyl alcohol within the threshold region of narcosis to be 9%. Above this level, alcohol acts as a narcotic, i.e., it reduces the muscle in a short time to a complete state of nonexcitability.

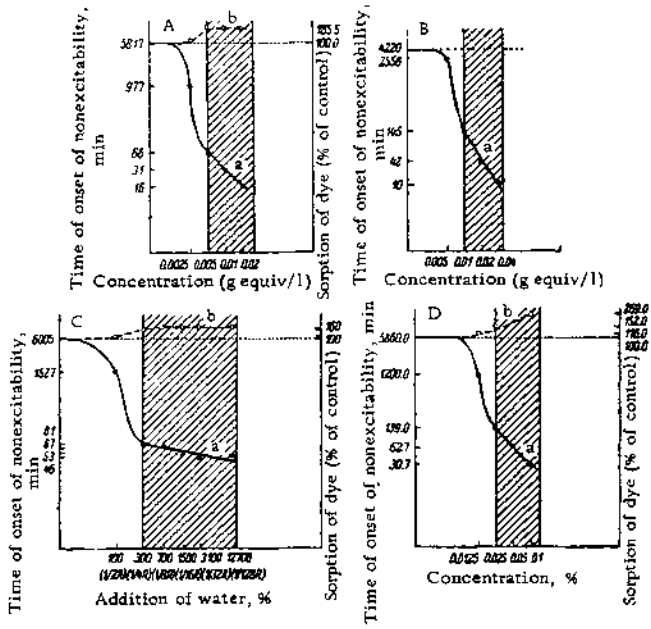


FIGURE 34. Relationship between concentration of the substance and (a) onset of nonexcitability; (b) change in staining of frog muscle

A—HCl; B—NaOH; C—dilution of Ringer's solution with water; D—quinine hydrochloride. Remaining legend—as in Figure 33.
(A—according to Nasonov and Suzdal'skaya, 1948; B—according to Suzdal'skaya, 1948; C—according to Gavrilova, 1948; D—according to Nasonov and Rozental', 1948).

Indeed, 72% alcohol causes narcosis after an average of 3.3 minutes.
61 Decreasing the alcohol concentration by half (36%) causes an increase in narcotization time by only 1.73 (5.7 minutes). On further halving of the concentration (18%), the time of onset of narcosis (9 minutes) increases 1.6 times. On additional twofold dilution (9%), time of narcotization (68 minutes) increases approximately sevenfold, but upon subsequent twofold dilution there is a great increase in the time necessary for complete loss of excitability. At this concentration (4.5%), the muscles survived on the average 7,632 minutes (127 hours), i.e., more than 100 times longer than in the previous concentrations, and somewhat less than the controls.

Figure 35, A, shows very clearly this steep threshold projection of the narcotization curve from 4.5% alcohol to 9% alcohol*.

---

* It is not difficult to understand that the threshold nature of the effect of macromolecular substances slowly diffusing into the depth of the muscle will be masked when whole muscles are used. In these cases either isolated fibers or very thin muscles consisting of two rows of fibers should be used.

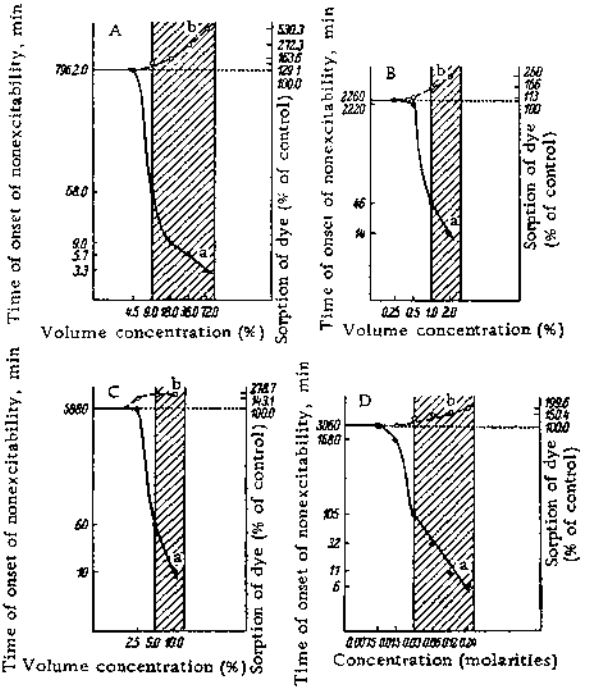


FIGURE 35. Relationship between the concentration of the substance and (a) time of onset of nonexcitability; (b) change in staining power of frog muscles

A—ethyl alcohol; B—Iso-amyl alcohol; C—ethyl ether; D—chloral hydrate; remaining legend as in Figure 33. (A, C—according to Nasonov and Suzdal'skaya, 1948; B—according to Nasonov, 1948a; D—according to Zelenkova, 1949).

The concentration of 9% corresponds to the threshold of the limit of adaptation. This is followed by a concentration area in which narcosis occurs very rapidly and is probably determined only by the velocity of denaturation of protoplasm proteins.

The subthreshold concentration of alcohol (4.5%) located on the very border of rapid decrease in excitability is of special interest. In our experiments the use of this concentration resulted in an approximately similar survival time of muscles as in the controls. Kiro (1954) who studied this zone, further observed that when muscles were taken from fresh frogs and immersed in subthreshold concentrations of certain narcotics (alcohol, urethane, chloral hydrate, and others) added to Ringer's solution, they lived longer than in pure Ringer's solution. The survival time of muscles in a subthreshold concentration of alcohol increased twofold as compared

to the control (Figure 36). The reason for this increased survival time is not yet clear. The investigation showed that the narcotic action of all the alcohol concentrations (except 72%) was reversible. Thus, if the damaging effect were assessed by the speed of onset of complete narcosis, ethyl alcohol acts as a damaging agent, starting from concentrations above 9%.

Table 10

Effect of ethyl alcohol on frog muscles (according to Nasonov and Suzdal'skaya, 1948)

Concentration per volume, %	Time of onset of nonexcitability-narcosis, min	Magnitude of contraction, mm	Increase in staining-paranecrosis, (% of control)
0	7962	0	0
4.5	7632	0	- 5.5
9.0	68	3.1	+ 29.1
18.0	9.0	9.5	+ 63.6
36.0	5.7	21.6	+ 172.3
72.0	3.3	22.2	+ 430.3

Remark: Each number in this and in the subsequent tables represents an arithmetic mean of the results of 5-12 experiments.

An attempt was made to assess the degree of paranecrosis due to varying concentrations of ethyl alcohol by the sorption method of dyes. This was not possible because alcohol interferes with the staining of living
62 and dead substrate, at precisely those concentrations at which these agents begin to act as narcotics (Nasonov and Aleksandrov, 1937). We were therefore forced to examine muscles treated with the alcohol solution, and subsequently rapidly rinsed with Ringer's solution. Under these conditions partial repair might have taken place, which had to be ignored, assuming that during this short interval the repair was minimal.

The results of these experiments are shown in Table 10 and in Figure 35, A (curve b). Ethyl alcohol in concentrations up to 4.5% did not affect the binding of the dye to any considerable extent (a certain decrease in staining at 5.5% was observed, which was however within the limit of error of the method). Starting at 9%, a progressive increase in staining occurs, indicating increasing damage of protoplasm with increased concentration of the solution.

Thus, if damage to living substrate were assessed by increased staining power, ethyl alcohol would be considered as a damaging agent and as a narcotic, starting from the same concentration (9%).

In order to find the concentration of ethyl alcohol at which it becomes
63 a physiological stimulant, experiments were performed on muscles on the basis that muscular contractions may be considered as a summation of stable, nonfluctuating local excitation.

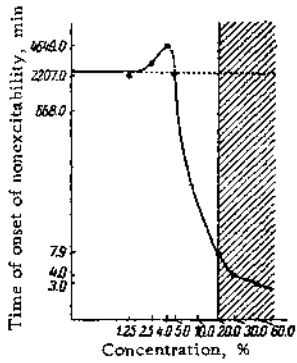


FIGURE 36. Relationship between the concentration of ethyl alcohol and onset of nonexcitability (according to Kiro, 1954).

The shaded area represents concentrations causing contraction. All the data are given in logarithmic scales

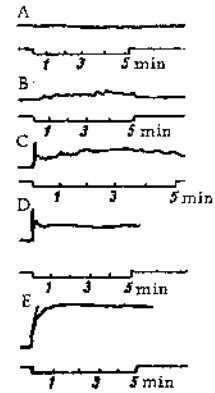


FIGURE 37. Action of various concentrations of ethyl alcohol on myograms of the sartorius muscle of the frog (according to Nasonov and Suzdal'skaya, 1948)

A—4.5%; B—9%; C—18%; D—36%; E—72%.

As in the experiments on binding of dye, alcohol of less than 4.5% did not cause any contractions. The first, very weak, but definitely clear-cut contractions appeared in all the experiments only at a 9% concentration (Table 10, Figure 37). Their mean maximum height, measured on the myogram, was 3.1 mm (at a ratio between the arms of the myograph lever of 1:5). Eighteen percent alcohol also caused contractions in all cases, but their average height was 9.5 mm. With 36% alcohol, the average height was 21.6 mm, and, finally, with 72% alcohol, 22.2 mm (Figure 37). The conclusions on comparing these data with those obtained in studying narcosis were that: on progressive increase in the concentration of ethyl alcohol, the threshold of its narcotic effect coincides exactly with the threshold of its effect as a stimulant causing contraction. With further increase in concentration, the rate of onset of complete narcosis and the magnitude of contractions increase in a parallel fashion. Starting from the same threshold, a progressive increase in paranecrosis of the living substrate was also seen.

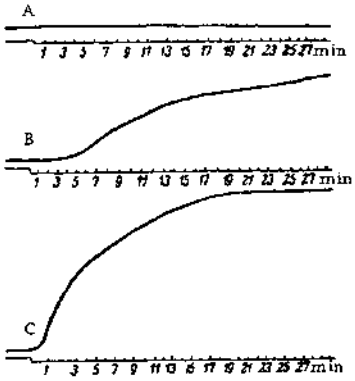


FIGURE 38. Effect of different concentrations of ethyl ether on myograms of the sartorius muscle of frog (according to Nasonov and Suzdal'skaya, 1948)

A—2.5%; B—5%; C—10%.

Further experiments were performed by the same technic, using ethyl ether. Concentrations of 10, 5, and 2.5% per volume (ether is insoluble above 10%) were used. The results are given in Table 11 and in Figure 35, C.

By observing the onset of nonexcitability, the threshold concentration (5%) was found. At this concentration, complete narcosis occurred on the average after 60 minutes (six times slower than in the next two higher concentrations). However, at a concentration 2.5 times lower the muscles lost excitability after 5,550 minutes, i.e., after a time period almost 100 times longer. Narcosis was freely reversible at all concentrations. As in the case of alcohol, no distinct contractions were observed below the threshold concentrations, but in all cases 5% ether caused clear contractions with no latent period, reaching a maximum after 10-20 minutes (Figure 38).

Thus, ethyl ether acted on muscles with the same regularity as alcohol. In both cases the threshold for narcosis coincided with that of contractions.

Experiments on staining after narcosis with ethyl ether were performed in exactly the same manner as with alcohol (Table 11). A strong increase in staining power (178.2%) was again observed at the threshold concentration (5%). The next concentration (10%) gave approximately the same effect. However, in the case of ether, the subthreshold concentration (2.5%) also caused a noticeable, although much weaker, increase in staining (43.1%).

To sum up, ethyl alcohol causes paranecrosis of the substrate even at subthreshold concentrations, but a sharp increase in paranecrosis is observed on approaching the threshold concentration or exceeding it.

The effect of a neutral salt (NaCl) will now be considered. The experiments were similar to those performed above. Ringer's solution with an increased concentration of NaCl served as the stimulant; the remaining components of the solution were not changed. Solutions containing the following concentrations of NaCl were employed: 0.65% (Ringer's solution (1R), control), 1.3% (2R), 2.6% (4R), and 5.2% (8R). The results are shown in Table 12 and in Figure 33B.

From the data in Table 12, it will be seen that the threshold narcotic concentration within the range employed was 2.6% NaCl (4R). Complete nonexcitability set in on the average after 48 minutes, while at a subthreshold concentration, the muscles lost their excitability only after 1,235 minutes, i.e., 26 times more slowly. The narcotic effect of all the solutions was easily reversible.

As in the case of alcohol and ether, the subthreshold narcotic concentration of NaCl (1.3%) never caused contraction, while at the threshold concentration (2.6%) a weak, but nevertheless clearcut contraction (4 mm

average) was observed in all the experiments. An eightfold concentration always led to powerful contraction, on the average eleven times greater than the threshold.

Table 11

Effect of ethyl ether on frog muscles (according to Nasonov and Suzdal'skaya, 1948)

Concentration per volume, %	Time of onset of nonexcitability-narcosis, min	Magnitude of contraction, mm	Increase in staining-paranecrosis, (% of control)
0	5880	0	0
2.5	5550	0	+ 43.1
5.0	60	59.5	+178.2
10.0	10	106.5	+178.7

Table 12

Effect of NaCl on frog muscles (according to Nasonov and Suzdal'skaya, 1948)

Weight concentration, %	Time of onset of nonexcitability-narcosis, min	Magnitude of contraction, mm	Increase in staining-paranecrosis, (% of control)
0.65 (1R)	5867	0	0
1.3 (2R)	1235	0	+ 10.6
2.6 (4R)	48	4.0	+ 32.1
5.2 (8R)	6	45.0	+122.4

65 The specific feature of the contractions caused by NaCl was that with their appearance, a variable number of individual twichings were observed which later disappeared. Dzhamusova and Ponomarenko (1954) showed that these twitchings are the results of individual impulses remaining in nerve fibers. If the frog muscles were previously denervated and peripheral sectors of the motor nerve were allowed to degenerate, there were no isolated twitchings on the myograms, although the smooth contractions appeared at the same concentrations without a latent period*. Experiments with staining have shown (Table 12 and Figure 33 B) that very weak paranecrotic changes (average increase in staining of 10.6%) were present at the

---

* Consequently, in cases where the agent used causes frequent separate twitchings of muscles it is important to elucidate whether these depend upon direct contraction of muscle fibers, or on stimulation of the remaining nerve fibers.

subthreshold concentration, while a strong increase in staining power began at the threshold (+ 32.1%) and increased to 122.4% at a concentration of 5.2%—8R.

Thus, as in the previous cases, the threshold of the narcotic effect of NaCl coincided exactly with the threshold appearance of contractions, i.e., with those concentrations at which NaCl began to act as a physiological stimulant, causing a state of stable excitation in the muscles. Paranecrotic changes of the substrate also appeared at subthreshold concentrations, increasing progressively with higher NaCl concentrations.

KCl is a final example of the action of a neutral salt. It is of interest because, like NaCl, it is an ingredient of Ringer's solution, although in much lower concentration. Its threshold concentrations are correspondingly lower. In order to study the effect of KCl, the same method was used as in the above series. Ringer's solution in which only the concentration of KCl was increased was employed as stimulant. The following series of concentrations was used: 0.15, 0.3, 0.6, 1.2, and 2.4%. The results are illustrated in Table 13 and in Figure 33 A, from which it will be seen that in the case of KCl, there was a clear threshold of narcotic action at a concentration of 0.3%.

The narcotic effect of potassium chloride was reversible at all the dilutions studied. Here, too, contractions appeared, beginning with the narcotic threshold dose, increasing progressively with higher concentration. It was characteristic of potassium-induced contractions that they occurred instantaneously, reaching a maximum after 1-2 minutes, and subsequently decreasing, sometimes to the initial level (Figure 40). As in previous cases, paranecrotic changes were present to a small extent at the subthreshold dose (increase in staining of 11% on the average), but reached a considerable value (+ 27%) at the threshold concentration (0.3%).

Thus, KCl acted like the other agents. It was a physiological stimulant, a narcotic, and an agent causing alterations in proteins, starting from the same concentrations. Finally, hydrochloric acid was diluted with
56 Ringer's solution, being partially neutralized by carbonate. Solutions of 0.02, 0.01, 0.005, and 0.0025 N concentrations were employed (Table 14 and Figure 34 A).

Under the given conditions of dilution the narcotic threshold of HCl was 0.005 N. Contractions observed at this concentration were of quite considerable height (33 mm average) and reached 88 mm on the average in 0.02 N solutions. Figure 41 clearly shows that all the contractions caused by HCl showed two peaks.

The experiments showed that a small increase in staining (+ 7.2%) was seen at subthreshold concentrations of HCl (0.0025 N), while the threshold concentration caused an increase of 87.8%.

Thus, the laws applicable to the action of ethyl alcohol, ether, NaCl and KCl on muscles, also apply to hydrochloric acid.

In addition to these substances, a further series of agents having a threshold effect were investigated. These included three bivalent chlorides-$CaCl_2$, $BaCl_2$, and $MgCl_2$ (Rozental', 1948); an alkali—NaOH (Suzdal'skaya, 1948b); sodium salts of Br and SO_4; and chlorides of Rb, Cs, and Li (Suzdal'skaya, 1952); hypotonicity, i.e., Ringer's solution deficient in sodium chloride (Gavrilova, 1948); quinine hydrochloride (Nasonov and Rozental', 1948); isoamyl alcohol (Nasonov, 1948a), and chloral hydrate

(Zelenkova, 1949). All these agents were of a different nature, and sometimes even of opposite physicochemical character (as, for example, alkali and acid, excess of NaCl and lack of it). But, as will be seen from Figures 32-35, the effect is the same; paranecrosis, narcosis, and stimulation take place in muscles with the same or very similar doses.

Table 13

Effect of KCl on frog muscles (according to Nasonov and Suzdal'skaya, 1948)

Weight concentration, %	Time of onset of nonexcitability-narcosis, min	Magnitude of contraction, mm	Increase in staining-paranecrosis, (% of control)
0	2760	0	0
0.15	1128	0	+11
0.30	38	28	+27
0.60	17	56	+30
1.20	7	94	+40
2.40	4	98	+42

Table 14

Effect of HCl on frog muscles (according to Nasonov and Suzdal'skaya, 1948)

Concentration in g equiv/1	Time of onset of nonexcitability-narcosis, min	Magnitude of contraction, mm	Increase in staining-paranecrosis, (% of control)
0	5817	0	0
0.0025	977	0	+ 7.2
0.005	66	33	+87.8
0.01	31	55	+85.5
0.02	16	88	+77.7

Notes: 1—In the present table, the concentrations of the acid are given without taking into account neutralization by carbonate.
2—For the determination of the magnitude of contraction, the first peak was measured.

The rule applies not only to chemical stimulants but to some extent to such physical agents as high hydrostatic pressure and sound. This is well seen in Figure 13, where there is a sharp increase in vital staining of muscles in the region of 85-100 db, while sonic contractions appear at 90 db.

67 According to Golovina (1955a), considerable increase in staining of muscles takes place in the zone of pressures of 400 atmospheres. According to Ebbecke and Hasenbring (1935) muscle contractions also appear at this pressure.

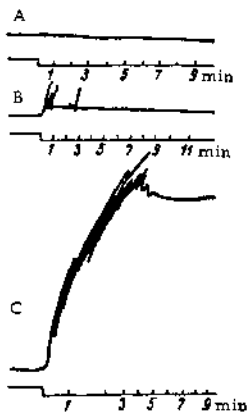


FIGURE 39. Effect of various concentrations of NaCl on myograms of the sartorius muscle of the frog (according to Nasonov and Suzdal'skaya, 1948)

69 A—1.3% (2R);
B—2.6% (4R);
C—5.2% (8R).

Very interesting data were obtained by Butkevich (1948), who treated frog muscles with increased temperature. She observed that muscle immersed in Ringer's solution at 33° C showed no traces of contraction (Figure 42). Only at 34° C did contractions appear, at first very weak, and later growing in intensity. Figure 43 shows rapid reduction in the time of onset of nonexcitability of muscles in this zone, i.e. a rapid development of thermonarcosis (refractivity) and an upward trend of the staining curve, indicating paranecrotic change in the protoplasmic proteins.

In parallel experiments, Butkevich determined the difference between the electric potentials of the heated and nonheated parts of the muscle. The results are illustrated in Figure 44, from which it can be seen that heating to approximately 30° C did not cause any noticeable difference in potential. Later, a certain shift in the direction of positive potential was observed in the heated part, which may be explained by purely physical conditions of the experiment. Still later, starting from 35° C, a steep rise of the curve is seen, completely corresponding to the region of muscular contractions and to the region of onset of refractivity.

The difference in potential might be considered as the injury potential, and as the negativity of the stimulated sector.

During an increase in intensity of a given stimulus acting on living substrate, firstly the threshold region will be reached at which the agent starts to act as a physiological stimulant. This causes muscle contractions. Nonexcitability or narcosis then appears, accompanied by paranecrosis in the protoplasm and electronegativity*. On further increase in the intensity, there is gradual increase in the magnitude of the contraction and of the velocity of onset of narcosis, likewise in the degree of paranecrosis and potential difference.

At first sight these conclusions may seem paradoxical. Instead of the expected sequence of excitation, narcosis, and damage (the rule of Arndt-Schulz), there is an almost simultaneous appearance of damage of the substrate, narcosis, and stable excitation increasing in direct proportion to the strength of the stimulus. In other words, excitation,

---

* Dzhamusova (1957), in our laboratory, similarly analyzed the effect of various substances on skeletal muscles, taking into account not only the concentrations of the substances with the same duration of action, but also the time the muscles were immersed in the solutions. She observed the same reactions expressed in even clearer form.

damage and narcosis are only outward manifestations of one and the same condition of the cell. They are not consecutive phases.

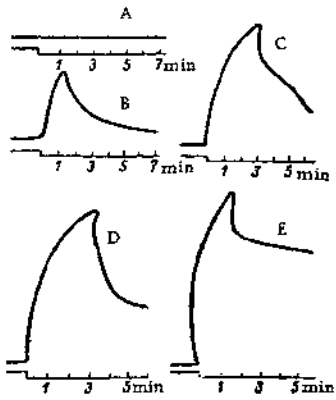


FIGURE 40. Effect of various concentrations of KCl on myograms of the sartorius muscle of the frog (according to Nasonov and Suzdal'skaya, 1948)

70 A—0.15%; B—0.3%; C—0.6%; D—1.2%; E—2.4%.

However, a typical excitation wave of muscle or nerve fiber is intimately linked with the wave of refractivity. If the excitation wave could be halted and the fiber studied simultaneously, it would be found in deep narcosis. Thus excitation and narcosis are not mutually exclusive. On the contrary, they are inseparably linked as alternatives of the same phenomenon. Generally, when muscle is narcotised it contracts. During muscle stimulation contractions occur under the influence of electric current. Simultaneously at this point, nonexcitability develops, or narcosis in the broad sense of this word (cathodic depression).

Customarily narcosis and excitation are regarded as opposites, since activity in any organism is thought to be due to alternation between the two. If a stable, nonoscillating stimulus (narcosis) is initiated within any nerve or muscle fiber or cell, the latter become nonconductive.

Excitation may precede narcosis, this being the basis of the Arndt-Schulz law. However, in relation to cell narcosis the term "increased excitability" should be used instead of "stimulation" or "excitation".

Thus, we consider that muscle narcosis is caused not only by typical narcotics, but also by any nonspecific stimulants, like refractivity of a nonoscillating stable stimulus, expressed externally by contraction.

Our conclusions regarding muscle contractions are very similar to, if not identical with, those on excitation and narcosis, formulated by the author of the theory of parabiosis, based on studies mainly of nerve conduction. Vvedenskii (1901) and later Ukhtomskii (1904) and Rusinov (1936) attempted to relate certain types of muscle contraction (i.e. idiopathic, etc.) with parabiosis of the nerve. These concepts were neither developed nor confirmed. More recently data supporting such a relationship were produced by Kvasov (1949), Ushakov (1952a, 1952b), Ushakov and Cherepanova (1952), and Zhukov (1956).

Vvdenskii's ideas have hitherto not been understood either abroad, or in the U.S.S.R., probably because his studies were based on a nerve which upon excitation did not produce any apparent external activity.

It is not so easy to agree that the parabiotic segment of the nerve, seemingly characterized only by negative attributes (loss of excitability, loss of ability to conduct impulses, etc.), is a stimulated sector. Many observers, even among the supporters of Vvedenskii's theory, accept this basic thesis in its relative sense, emphasizing that parabiosis is either "stimulation of a special kind", or "overstimulation".

The assertion that the narcotized sector of muscle is in a state of stable excitation is much more obvious. Indeed, if the observed rule is

true, any attempt to cause contraction of the muscle by any agent whatsoever will result in a state of block. However, Ushakov (1949) has shown that if conduction of impulses through a narcotized segment of the muscle is studied, all the typical stages of change in conduction characteristic of nerve parabiosis can be observed.

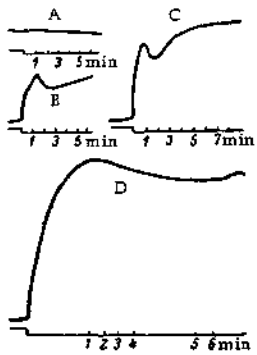


FIGURE 41. Effect of various concentrations of HCl on the myograms of the sartorius muscle of the frog (according to Nasonov and Suzdal'skaya, 1948)

A—0.0025 N; B—0.005 N; C—0.01 N; D—0.02 N.

The objection to Vvedenskii's work (especially from a pharmacological viewpoint) is that while demonstrating the same reaction of the nerve to entirely different agents, he disregarded their specific properties. In the case of muscles, this specificity of stimulants is clearly manifested. The muscle reacts to all the agents, and always obeys the same law, according to which the threshold region of excitation coincides with the threshold region of narcosis and paranecrosis.

The myograms show that each agent has a characteristic record; a rapid and low contraction with no latent period, caused by alcohol; a powerful, slowly increasing contraction with a latent period caused by ether; a double-peaked contraction curve caused by acid; frequent twitchings due to NaCl; rapid maximum contraction with subsequent rapid drop caused by KCl, etc. (Figures 37-41). It is not the aim of this work to search for the sources of these specific properties or the general nonspecific background. The reader is referred to the work of Aleksandrov (1948). Apart from the irreversible changes mentioned, the whole cycle could proceed till the death of the tissues involved without specific differences.

At first we considered paranecrotic changes of living substrate as reversible damage. Later, we were convinced that paranecrotic changes may be detected even at subthreshold strengths of the stimulant, increasing rapidly with an increased dose. At the same time, the natural physiological contraction of muscles, according to contemporary theories, may be considered as a transient electric (cathodic) contraction, which, like other forms of contraction, ought to be accompanied, at least initially, by paranecrosis.

Is it correct, then, to define paranecrosis as reversible protoplasmic damage?

Damage usually implies a pathological process. In this sense, any local stimulation of muscle fibers, including electric stimulation, would be a pathological phenomenon, which of course is not so. Therefore paranecrosis is best described by the more neutral term, "reversible alterations".

What is the relationship between paranecrotic changes of the living substrate and contraction?

Paranecrosis appears in any cell following application of varied stimulants. There is some basis for believing that these changes are due to reversible denaturation of protoplasmic proteins, the latter being a fundamental result

of interaction between the stimulants and the cell. In a phylogenetic sense this is probably one of the most ancient properties of the cells. But these changes serve as a release mechanism for a chain of complex biochemical processes, resulting in cellular activity. In the case of muscle fibers, this activity is expressed as mechanical work.

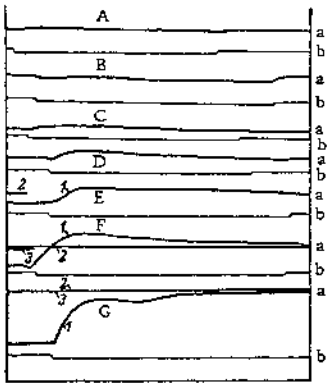


FIGURE 42. Contractions of the sartorius muscles of the frog due to high temperatures (according to Butkevich, 1948)

A--32° C; B--33° C; C--34° C; D--35° C; E--36° C; F--37° C; G--39° C.

a—contraction of muscle; b—mark of stimulation. The numbers designate the sequence in recording the contractions.

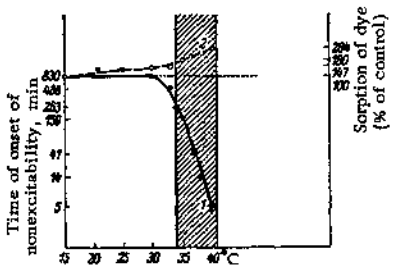


FIGURE 43. Effect of high temperatures on sartorious muscles of the frog (according to Butkevich, 1948)

1—Time of onset of nonexcitability; 2—Sorption of dye. The shaded area designates the range of temperature causing contraction. The data in both ordinates are in logarithmic scales.

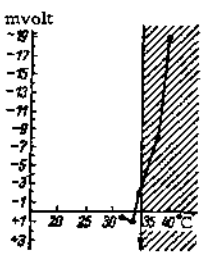


FIGURE 44. Potential difference between heated and nonheated surfaces of muscle due to temperature (according to Butkevich, 1948) The shaded area designates the temperature range causing contractions.

The ability of connective tissue cells to contract under the influence of different stimulants was discovered by Kühne (1864), who studied the fibrocytes of the cornea. He observed a reversible retraction of appendages, and a rounding-off of cell bodies and their nuclei following the action of mechanical factors, e.g. electric current and nervous stimulation.

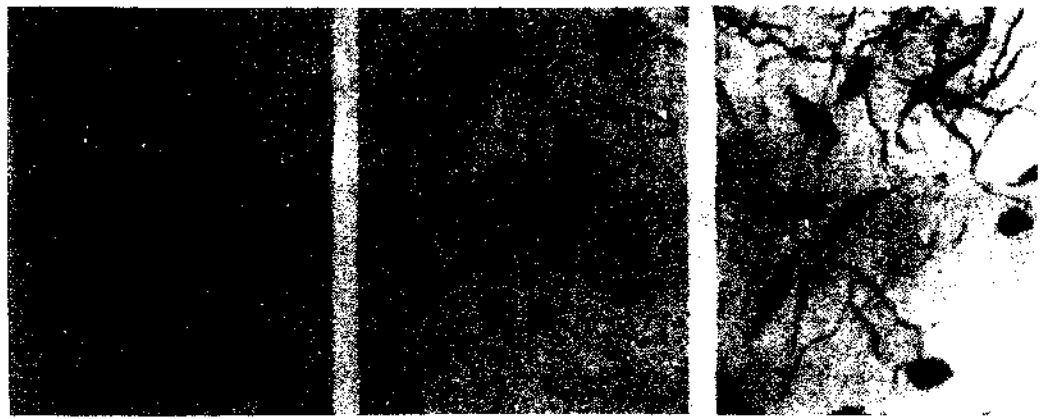
Möllendorff et al. (Möllendorff and Möllendorff, 1926; M. Möllendorff, 1927; W. Möllendorff, 1927; Knake, 1927; Stockinger, 1927) investigated fibrocytes of loose connective tissue and skin following the action of ultraviolet rays, injection of foreign serum, trypan blue, suspension of china ink, etc. According to these authors, the nuclear-cytoplasmatic sectors of the fibrocyte reticulum withdraw their appendages and isolate themselves from the reticulum. They lose their connection with the neighboring elements, which they transform into histiocytes, or macrophages and granulocytes.

Later, Yasvoin (1930) observed rounded fibroblasts of skin in the region of aseptic inflammation caused by burns and introduction of foreign bodies. Seelich and Stockinger (1953) using fibroblast cultures observed a rounding off and contraction of cells following the action of tannin, antiserum, and Janus green.

The experiments of Vol'fenzon (1954) and of Aleksandrov and Vol'fenzon (1956) on fibroblasts of the subcutaneous loose connective tissue, are of special interest. Vol'fenzon (1954) studied the effect of novocaine on vital staining of cells from various tissues. The dye used was an 0.025% solution of neutral red in Ringer's solution. In all the cells studied (nerve cells, glandular cells of the pancreas, epithelium of kidney tubules, and fibroblasts) paranecrosis took place at approximately the same concentration of novocaine (from 0.5 to 2%), as that used for local anesthesia in medical practice (0.25-2%).

This concentration of novocaine also caused an easily reversible contraction of fibrocytes. Thus, after a one-hour exposure to 1% novocaine (in Ringer's solution), the cells retracted their appendages and rounded themselves off, returning to their initial form after removal of the novocaine (Figure 45, B). Thus, with novocaine too, the concentrations causing contraction (local stimulation) and paranecrosis are more or less the same.

In other studies by Aleksandrov and Vol'fenzon (1956), a quantitative method of measuring cell contraction was used. The authors showed that like muscle contraction, this is nonspecific, and may be caused by varied chemical agents. In rabbits killed by air emboli, sections of subcutaneous cellular tissue from the spinal region were placed in Petri dishes for 20-30 minutes with Ringer's solution at 37° C. Subsequently, one part of the section was transferred to vessels containing solutions of other substances prepared in Ringer's solution, while the control was left in pure Ringer's solution. After 30 minutes, the experimental and control films were carefully spread on small pieces of cardboard, fixed in 10% formalin, rinsed
73 and stained with hematoxylin lacquer according to Yasvoin, and mounted with Canada balsam. The decrease in the maximum length of the cells was measured. For this purpose, a certain number of cells in the preparation were drawn on paper, and the greatest distance between the tips of the cell bodies was measured on the drawing by a ruler.



A B C

FIGURE 45. Action of 1% novocaine on connective tissue cells of rabbit (subcutaneous cellular tissue—according to Vol'fenzon, 1954)

A—Control; B—One-hour exposure to novocaine (at 37° C); C—Three-hour rinsing in Ringer's solution after the action of novocaine (at 37° C). Fixation with 10% formalin. Staining with hematoxylin lacquer, according to Yasvoin. Phase contrast microscope (objective X 60, ocular X 5).

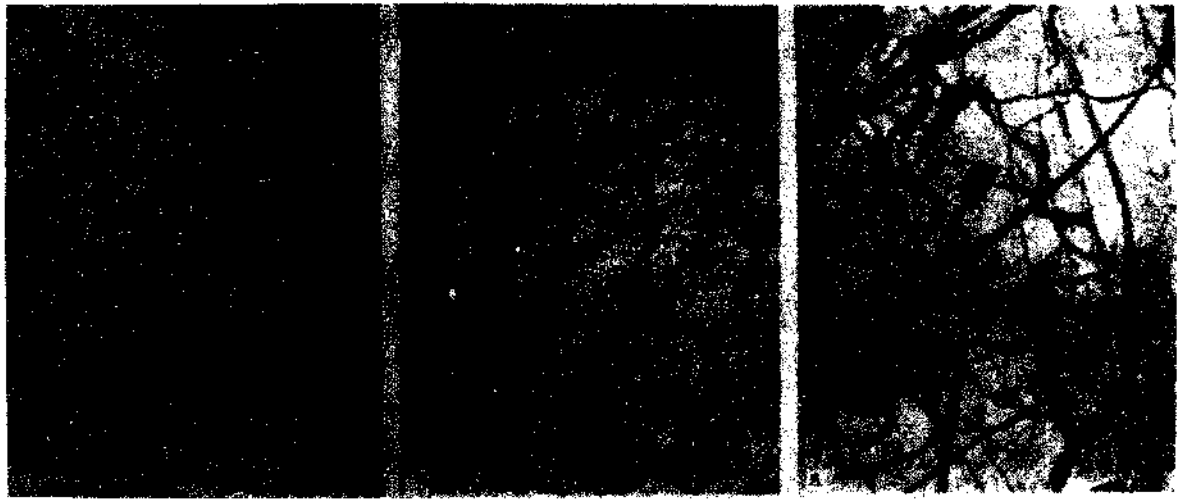


FIGURE 46. Action of 5% ethyl alcohol on a section of subcutaneous cellular tissue of rabbit (according to Aleksandrov and Vol'fenzon, 1956)

A—Control; B—30-minute action of alcohol; C—2-hour washing in Ringer's solution after the action of alcohol. Fixation with 10% formalin. Staining with hematoxylin lacquer according to Yasvoin. Phase contrast microscope (objective X 20, ocular X 15).

Table 15

Change in length of fibrocytes after exposure to ethyl-alcohol solutions of different concentrations (according to Aleksandrov and Vol'fenzon, 1956)

Experiment No.	Treatment	Number of cells	Length, μ	Length (% of control)	Mean error, %
1	Control	359	25.9	100	± 2.0
	Alcohol 10%—	418	19.7	76	± 1.6
2	Control	274	33.4	100	± 0.7
	Alcohol 10%—	372	16.4	49	± 0.7
3	Control	53	36.2	100	± 5.7
	Alcohol 5%—	46	25.5	71	± 4.0
	" 10%—	72	20.9	58	± 1.6
	" 30%—	111	34.9	96	± 3.6
4	Control	447	27.9	100	± 2.1
	Alcohol 5%—	120	24.8	89	± 3.3
	2 hours in Ringer's solution after the action of 5% alcohol (reversibility)	180	26.3	94	± 2.7
5	Control	174	36.6	100	± 3.2
	Alcohol 5%—	174	19.5	53	± 1.0
	2 hours in Ringer's solution after the action of 5% alcohol (reversibility)	174	31.3	86	± 2.0
	Alcohol 10%—	250	16.1	44	± 0.6
	2 hours in Ringer's solution after the action of 10% alcohol (reversibility)	226	25.3	69	± 1.8

Figure 46 B shows fibrocyte contractions caused by 5% alcohol, and their return to normal after removal of the agent (Figure 46 C). Table 15 gives the numerical results of the experiments in the form of arithmetic means, the probable errors being calculated on the basis of hundreds of measurements.

From the data in Table 15 it will be seen that as in the case of muscular contractions, the effect is gradual, i.e., the magnitude of contraction is quantitatively dependent on the concentration of the substance (experiments no. 3 and 5). In experiment no. 3 however, this was only true for alcohol concentrations below 10%. A concentration of 30% caused smaller contractions, probably because irreversible fixation of the tissue developed earlier than the physiological response reaction. In 5% and 10% dilutions, the cell contractions were reversible, more completely so in the former dilution (experiments no. 4 and 5).

Similar experiments were performed by the authors with novocaine, $CaCl_2$, KCl, adrenalin and trypan blue. The threshold concentrations were: alcohol 5%, novocaine 0.5%, $CaCl_2$ 0.25%, KCl 0.5%, and adrenalin—1:100,000. In addition, during the course of the experiments, it was shown

74 that simple immersion of the tissue in Ringer's solution caused contraction. Such contraction was also observed in muscles kept outside the organism (Ushakov and Dzhamusova, 1954).

Contraction of connective tissue cells was also accompanied by rounding of their nuclei. This fact was described by Kühne (1864), who, however, considered it as a purely passive phenomenon.

The facts regarding contraction of fibrocytes show a great similarity to the data on muscular contractions. Indeed, in both cases, there is firstly a mechanical contraction of stretch-out formations, and secondly a nonspecific response reaction, in which different agents in their various physical and chemical agents cause the same physiological response of the cell. Finally, in both cases, these responses appear simultaneously with paranecrosis of the protoplasm.

Contraction is a function characteristic of mesenchymal tissues, to which group belong not only the different types of connective tissue, but also smooth muscles. In fibrocytes, however, much more primitive and ancient forms of contraction take place. This is seen by comparing the velocity of contraction. In skeletal muscles, this is measured in fractions of a second. In fibrocytes, according to W. Möllendorff (1927), a complete rounding of the cells is observed in the vicinity of the site of injection only 15 minutes after repeated introduction of foreign serum. However, Kühne obtained strong compression of cornea cells only 2 minutes after stimulation with electric current.

There is no doubt that the reactive contractions of fibrocytes must be considered as a very primitive form of local cellular stimulation, similar to both ameboid and muscle contractions.

Metabolism during Local Excitation

In the previous subchapters, we tried to show that stimulated tissues enter a state which may be regarded as local stable excitation (Vvedenskii's parabiosis). In this respect interesting effects are observed in skeletal muscles at the site of action of the stimuli. The muscle begins to lose its excitability (narcosis, refractivity) and at the same time, a local stable contraction takes place. In order to prove that in the given case, the "local reaction" may really be considered as "local excitation", it would be of interest to compare the metabolism during tetanic contraction of muscle (when it is undoubtedly in a stimulated condition), with the metabolism of the muscle during normal contraction.

Gasser (1930), cites the literature on this problem, but this report is somewhat dated, since it precedes the work of Lundsgaard (1930a-1930c,
75 1934). At that time the importance of phosphorus-containing high-energy compounds which split during muscular metabolism was not yet understood by biochemists. This literature mainly deals with glycogen catabolism, accumulation of lactic acid, and release of heat. Nevertheless, on the basis of the reports at his disposal, Gasser concluded that in muscular contractions heat production and some aspects of metabolism were similar to tetanus.

More recent investigations on these problems were carried out by Deuticke and Ebbecke (1937), and Panteleeva (1953). The former made a

thorough investigation of metabolism during muscular contractions caused by high pressure. The study of metabolism in this type of contractions is of special value because under high pressure, no extraneous substances are introduced which may distort the normal course of metabolic reactions.

The authors of the above-mentioned paper considered these contractions to be the most physiological of all. They especially emphasized that hydrostatic pressure as a stimulant acts simultaneously and uniformly on the whole muscle fiber, which is not true for such stimulants as mechanical, thermal, chemical, or osmotic.

The threshold pressure is 200-250 atmospheres. After further increase of pressure the magnitude of contractions is quantitatively proportional to the pressure. Deuticke and Ebbecke investigated metabolism under 300 and 500 atmospheres. Muscles were subjected to pressure for various lengths of time from 0.5 to 30 minutes, after which they were rapidly removed from the pressure chamber, and transferred to liquid air for freezing. This procedure lasted 25-50 seconds. Subsequently, the muscles were minced, and the mince was analyzed by ordinary methods for phosphocreatine, glycogen, lactic acid and inorganic phosphates. The results of these studies are described in Figures 47 and 48.

Frog muscle always responded to a pressure of 300 atmospheres by contraction. After removing the muscle from the pressure chamber, it resembled the external appearance of the control and showed no traces of residual contraction.

The creatine phosphate content of the muscle decreased noticeably, as in the case of normal tetanic contraction (Figure 47), with the exception of the first 30 seconds of action, during which creatine phosphate decrease was not observed. However, it is possible that after such a shortlived stimulation, resynthesis of this substance took place during removal of the muscle from the pressure chamber, a synthesis which can also take place under unaerobic conditions. After 3-4 minutes, one-third of the total creatine phosphate was split, while after 30 minutes the amount of this substance decreased twofold.

Under 500-atmosphere pressure, the muscle always reacted by strong contractions, terminating only after removal of pressure, when residual shortening was sometimes observed. In this case, after 5 seconds, one-third of the amount of creatine phosphate had disappeared, i.e., somewhat
76 less than after isotonic tetanus of a 2-4 second duration. In this case, as in the case of a 300-atmosphere pressure, after the period of initial rapid decomposition, the process is somewhat slowed down. Deuticke and Ebbecke are of the opinion that this phenomenon may be compared with tetanic contraction of muscles.

During normal muscle contraction transformation of glycogen and lactic acid is closely linked to the resynthesis of creatine phosphate. Figure 47 shows that at a pressure of 300 atmospheres, the amount of glycogen
77 after 20 minutes changed very little. The amount of lactic acid, however, invariably increased, although not strikingly. A stronger pressure (500 atmospheres) led to a considerable decrease in the amount of glycogen after 10-30 minutes, and to an increase in the lactic acid content (Figure 48).

Very interesting data were obtained on the amount of insoluble proteins. In his earlier studies, Deuticke (1930-1932) showed that after indirect tetanic stimulation of muscle under aerobic conditions, the solubility of the extracted proteins decreased in relation to the intensity of work

performed. This decrease was earlier explained by the authors as an expression of physicochemical processes in proteins of the myosin fraction. These observations are of special interest, since the insolubility of proteins, due to the influence of various agents, is one of the most characteristic symptoms of their denaturation. Deuticke's findings may thus serve as additional proof of the denaturation theory of excitation, and comparable with our data on the increase in vital staining.

The same decrease in solubility of proteins takes place on contraction due to compression, as in normal tetanus. This is observed 20 minutes after exposure to pressure of 300 atmospheres, while at 500 atmospheres, the solubility decreased by 20% in 5 minutes.

Deuticke and Ebbecke investigated the inorganic phosphates and other phosphorus compounds after muscular contraction, in comparison with the corresponding reactions in tetanus as seen in their own data and that in the literature. The comparison showed that the reactions following muscular contraction are the same whether the stimulus is via the nerve, or by compression.

Deuticke and Ebbecke therefore concluded that there is no substantial difference between muscular contraction caused by high hydrostatic pressure, and normal tetanic contraction. The difference is simply that in tetanus a number of separate stimuli, with their resultant reactions, are consecutive, while on compression the muscle is subjected to continuous stimulation. Therefore it enters a state of prolonged nonoscillating excitation and activity.

It is interesting that the "all or none" law is not applicable to contraction, but there is a quantitative relationship between the strength of the stimulus and the mechanical effect, as well as between the latter and the intensity of the biochemical processes (see Figures 47 and 48).

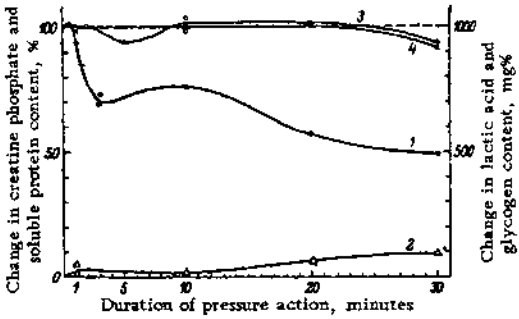


FIGURE 47. Effect of 300 atmosphere pressure on the content of (1) creatine phosphate, (2) lactic acid, (3) soluble proteins, and (4) glycogen in frog muscle (according to Deuticke and Ebbecke, 1937)

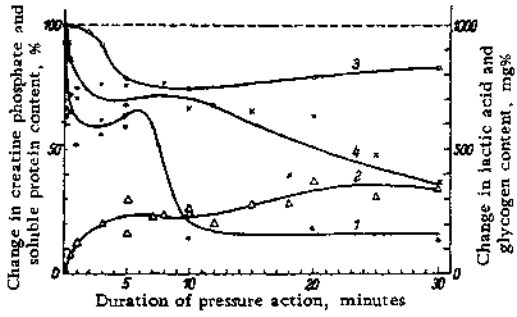


FIGURE 48. Effect of 500-atmosphere pressure on the content of (1) creatine phosphate, (2) lactic acid, (3) soluble proteins, and (4) glycogen in frog muscle (according to Deuticke and Ebbecke, 1937)

Panteleeva (1953) studied the metabolism of phosphorus compounds in tetanus, tonus, and contractions of skeletal frog muscles*. Tetanic
78 contractions were caused by stimulation of the nerve with induction current. Tonus-like contraction of the muscle was achieved by Zhukov's method (1936b, 1947), of stimulation of the nerve with simultaneous blocking by constant current. According to Zhukov, at a certain stage of polarization, only the tetanic response is blocked and the muscle responds to a series of impulses, passed through the nerve by a prolonged "viscous" contraction with no signs of fatigue. Finally, muscles were immersed in solutions of acetylcholine, chloral hydrate, quinine sulfate, and urea, as was described earlier for the sartorius muscle (Nasonov and Suzdal'skaya, 1948). After stimulation, the muscles were frozen in liquid air and ground into a fine powder. The inorganic phosphorus, creatine phosphate and adenosine triphosphoric acid (ATP) content were determined by the usual methods. Fifteen to sixty seconds after stimulation through the nerve, the amount of creatine phosphate decreased by an average of 32%. These results were in accordance with those from the literature.

However, an entirely different picture was observed in tonic contraction. Here, the creatine phosphate content varied in either direction, notwithstanding the fact that some experiments continued for 1,000 seconds. The arithmetic mean of experiments was expressed by a statistically insignificant number (+4.9). The same results were obtained for the decomposition of creatine phosphate in acetylcholine contraction. The problem of where the muscles derive energy for these forms of contraction was not investigated.

Panteleeva's data on metabolism in nonspecific contractions caused by chloral hydrate, quinine sulfate, and urea are very similar to those obtained in natural tetanic contraction. In all these cases, creatine

---

* The work of Deuticke and Ebbecke (1937) was obviously unknown to Panteleeva, since this author did not mention it.

phosphates split, with an intensity directly proportional to the concentration of the stimulant and its time of action, i.e. the same pattern as in contractions due to hydrostatic pressure.

Thus, in terms of metabolism of phosphorus compounds, nonspecific contractions are most closely related to normal tetanus. This ought to have been the conclusion following the data given by Panteleeva. But in fact she writes as follows: "Contractions caused by the action of chloral hydrate and quinine sulfate on muscles are accompanied by a clearly expressed splitting of creatine phosphate and adenosine triphosphoric acid, and an increase in the amount of inorganic phosphorus" (page 129). However, later on, the author makes a reservation, which in our opinion does not follow from the data given: "This splitting is the result of a pathological effect of the above-mentioned agents on the muscle, and is not a source of energy for the maintenance of contraction". The arguments cited by Panteleeva to support this point of view are unconvincing.

In general, her work is of interest, the more so since it is in complete agreement with the data of Deuticke and Ebbecke.

Finally, we shall discuss the work of Kondrasheva (1954) on metabolism of muscles in a state of parabiosis due to the action of various local stimulants. It must be noted, however, that Kondrasheva was obviously not familiar with the data of Deuticke and Ebbecke, nor with that of Panteleeva. Nevertheless, she reached the same conclusions.

Her studies were made on cat muscles, the vessels of which were
79 perfused with Tyrode solution containing different concentrations of the investigated substances. Some of these (phenamine, caffeine, adrenalin, sodium bicarbonate, sodium hydroxide, and others) caused a biphasic change in muscle excitability. At first, this increased (phase one); subsequently it almost disappeared (phase two). Other substances like 2, 4-dinitrophenol, methylene blue, sodium azide, sodium chloride, and hydrochloric acid caused an immediate decrease in excitability.

The content of inorganic phosphorus, creatine phosphate, ATP, and adenosine diphosphate (ADP) of the muscles was studied. The results are given in Figure 49 and in Table 16.

From the data in Table 16, it will be seen that during the first phase (increased excitability), a clearcut increase in organic phosphorus compounds (creatine phosphate and ATP) is seen relative to inorganic phosphorus. This indicates a predominant synthesis of high energy phosphorus compounds. In the second phase (decreased excitability) splitting of these compounds predominates.

Thus, after stimulation of skeletal muscles with nonspecific, and entirely foreign agents, a stable contraction (parabiosis), occurred at the site of application of the stimulant, with the same chain of biochemical transformations as in the natural tetanic contraction of muscles.

The question arises as to what happens to the sector of the nerve which is stimulated, bringing the nerve to a state of parabiotic block.

80 Many papers have been devoted to the metabolism of the physiologically stimulated nerve, studies of its respiratory metabolism, as well as its carbohydrate-phosphorous metabolism. The general literature pertaining to this problem will not be reviewed here. But it should be noted that in the stimulated nerve, the same basic biochemical processes are observed as in muscles, although on a much smaller scale. Thus, Gerard (1932), in his review stated that if at the moment of stimulation, muscle increased

its metabolism 1,000-fold, the nerve increased its metabolism only two-fold. Nevertheless, during the activity of the nerve, the same events occurred as in muscle. The oxygen uptake and production of carbon dioxide increased, lactic acid accumulated, the amount of creatine phosphate, and to some extent of ATP decreased, while the amount of inorganic phosphates increased.

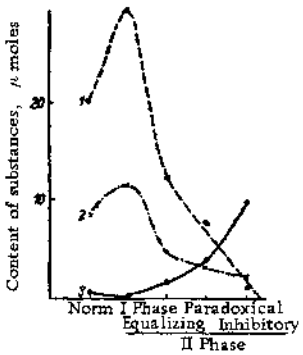


FIGURE 49. Content of (1) creatine phosphate, (2) ATP, and (3) ADP of muscles of the cat at various stages of parabiosis (in micromoles per gram of wet weight) (according to Kondrasheva, 1954)

That these changes are small by comparison with muscle is explained by the fact that conduction in the nerve produces a very small amount of electricity, while the active muscle, in addition to this relatively small expenditure of energy, produces a great amount of mechanical work. According to Gerard and Tupikova (1938-1939), only 15% more inorganic phosphate can be detected in the stimulated nerve than in the resting one; the creatine phosphate content drops on the average by 5%, while in the majority of cases the amount of ATP decreases only a little. But there can be no doubt that splitting of phosphorus-containing compounds is the direct source of energy for the stimulated nerve. Thus, according to Ronzoni (1931), on complete exclusion of glycolysis with free access to oxygen, the nerve maintains its ability to conduct (at the expense of splitting creatine phosphate), whereas this property disappears as soon as the creatine phosphate is exhausted.

Thus, two different forms of cellular activity have the same energy source and similar biochemical reactions. There is considerable evidence to support the contention that in addition to muscles and nerves, other tissues, such as liver, kidney, brain, have same basic reactions (Bolduin, 1949). Quite considerable amounts of phosphogen, for example, are found in spermatozoa. There are indications that these cells, as well as ciliary epithelium, obtain energy necessary for motion from a typical glycolytic reaction with the participation of ATP and creatine phosphate (Aleksandrov and Arronet, 1956). It is highly probable that the basis for vital activity of most different cellular elements is a variation of the same nonspecific chain of biochemical transformations, used by different cells as an energy source for various types of activity.

After local stimulation of the nerve, the questions arise firstly whether it enters a state of nonconductivity, i.e., of local narcosis; secondly, whether biochemical reactions are inhibited, as in nerve excitability, and thirdly, whether the local effect of nonspecific stimulants releases a characteristic chain of chemical transformations, as in muscles.

Few studies of these problems have been made. Misheneva (1955) thoroughly investigated carbohydrate-phosphorus metabolism in a nerve segment brought into a condition of nonconductivity by agents such as high
81 or low temperature and solutions of ether and chloroform. In all cases, the effect of the agents was reversible. This fact was checked not only by

restoration of conductivity to the narcotized sector of the nerve, but also by biochemical indexes.

According to Misheneva, temperatures up to 36° C do not substantially affect the content of phosphates and lactic acid of the nerve. However, higher temperatures cause sharp biochemical changes (Table 17).

Table 16

Ratio of the total organic phosphorus content of the acid soluble fraction, to the content of inorganic phosphorus at different stages of parabiosis in muscles (according to Kondrasheva, 1954)

Form of experiment	Norm	Phase I	Phase II		
			Equalizing phase	Paradoxical phase	Inhibitory phase
With nerve section	0.98 ± 0.11	1.55 ± 0.18	0.66 ± 0.05	0.59 ± 0.02	0.16 — 0.31
Without nerve section	2.5 ± 0.07	4.3 ± 0.35	1.8 ± 0.15	1.07 ± 0.08	0.35 — 0.88

Table 17 shows the effects of a nerve block caused by applying a temperature of 45° C for 30-60 seconds. Creatine phosphate split to an extent of 38.3%. The amount of inorganic phosphate increased by 36%. The ATP content remained unchanged. During a block of 2-3 minutes, the lactic acid content increased by 18.3%. With a block of 5-6 minutes, the amount of lactic acid increased twofold. This fact indicates that the formation of lactic acid took place during the blocked condition, and was not linked with any earlier stages of parabiosis.

82 During recovery of the nerve sector from a heat block, its phosphate and lactic acid content returned to the initial level, indicating reversibility of the phenomena studied.

Thus, complete biochemical recovery took place. A small difference (several percent) between the initial and the final conditions lay within the range of error of the method of study used.

Misheneva studied the metabolism of a nerve segment brought to a state of nonconductivity by decrease of temperature from —8 to —14° C (Table 17). The biochemical effect, in cold parabiosis (block) of the nerve, was the same as in heat parabiosis. The inorganic phosphate content increased by 40%. The creatine phosphate content dropped by 27.9%, and ATP by 19%, due to splitting. The same was true of lactic acid. As in heat parabiosis, the amount of lactic acid increased considerably, and after the nerve reverted from nonconductivity, its initial content of lactic acid was restored.

Misheneva studied the biochemical changes taking place in a nerve sector narcotized (by ether diluted 1:20) to a state of complete nonconductivity (Table 17).

In this case too, in a state of deep narcosis, the same shifts in phosphate content took place, as in blocks due to high or low temperature. The inorganic phosphorus content increased by 32%, while creatine phosphate

Table 17

Phosphate and lactic acid content (in mg percent of wet weight) of twin nerves of frog, (a) under normal conditions and (b) during block caused by different factors. Arithmetic means from 5-8 experiments (according to Misheneva, 1955)

Substance	Norm	Increased temperature		Norm	Recovery of heat block	Norm	Low temperature		Norm	Ether		Norm	Chloroform	
		Block caused by temperature of 45°C for 30-60 sec	Changes, %				Block caused by temperatures from −8° to 14° for 40-90 sec	Changes, %		Block caused by ether (1:20) for 30-70 sec	Changes, %		Block caused by chloroform (1:40) for 30-70 sec	Changes, %
Inorganic phosphorus	5.5	7.5	+36.0	4.9	4.9	4.7	6.6	+40.0	4.7	6.2	+32.0	6.3	8.2	+30.0
Creatine phosphate	4.7	2.9	−38.3	5.2	4.8	5.6	4.1	−27.9	4.2	2.	−30.0	4.7	2.6	+44.7
ATP	3.0	3.0	0	2.5	2.4	2.1	1.7	−19.0	2.9	2.9	0	2.3	2.2	− 5.0
Lactic acid,	35.8	42.8 (block in 2-3 min)	+18.3	38.8	39.3	35.6	46.2	+31.3	38.9	48.2	+26.9	38.5	52.2	+37.2
Lactic acid	32.7	45.7 (block in 5-6 min)	+40.2											

dropped by 30%. The amount of lactic acid increased to the same extent as in the narcotic action of high and low temperatures.

The same phenomena occurred on blocking conductivity by chloroform, diluted 1:40 (in Ringer's solution), acting for 30-70 sec. As in all the previous cases of stimulant action, the amount of inorganic phosphate in the nerve increased, the creatine phosphate content decreased, and lactic acid accumulated. All these changes were reversible. After restoration of nerve conductivity, the content of all the substances returned to normal.

These data are of exceptional interest. They show that in the case of the nerve too, such varied stimulants as high and low temperature, and narcotics, release the same chain of biochemical reactions as in normal physiological activity of the nerve. It is especially interesting that among the agents stimulating metabolism, the typical narcotics must also be included.

It used to be thought that narcotics necessarily inhibit all vital functions, including metabolism. Therefore the assumption that a typical narcotic given in a dose leading to deep narcosis stimulates metabolism, may seem paradoxical. It appears, however, that narcotics do not differ from other
83 true stimulants, e.g. high temperature, in their ability to stimulate glycolytic catabolism. In their ability to inhibit excitability of nerves, narcotics, in this respect, resemble other stimulants, since any one of them, at the site of its spplication, causes nonconductivity of the nerve or the muscle fiber (refractivity, narcosis).

It may be assumed that partially stimulated catabolism may correct the changes in protoplasm caused by the stimulus, and secondly the lowered excitability of the nerve fiber. This is in accordance with the hypothesis that reversible damage may be considered as the most ancient and general form of excitation.

Biochemical studies of catabolism at the site of application of a stimulus in nonconducting tissues are very scarce. There is some interesting work by Smoilovskaya (1938), but somewhat differently designed. She stained various surviving tissues of mammals (liver, kidneys, and lungs of rat, rabbit, and guinea pig) with neutral red. When these tissues were kept in Ringer's solution, the author observed the appearance of granules on the background of the unstained cytoplasm, and an optically empty nucleus. Ringer's solution with a lowered NaCl content was used as a stimulant (or reversibly damaging agent). (It was mentioned above that half-strength Ringer's solution did not cause contraction of frog muscle, and that only at quarter-strength was it a stimulant. This dilution, therefore, was the threshold one).

In Smoilovskaya's experiments with epithelia of mammals, twofold dilution of the salt solution also did not change the normal character of staining. After further dilution, granule formation disappeared, and the cytoplasm and the nuclear structures began to be stained. The process was easily reversible if the tissue was not kept too long in the hypotonic solution.

Simultaneously, Smoilovskaya investigated the leakage of phosphates from the tissues. It was shown that with half-strength solution leakage of phosphates was very small and only upon higher dilution did it increase. It is highly probable that in epithelial tissues too, nonspecific stimulants at threshold doses stimulate splitting of high-energy phosphorus compounds.

Recently, Braun (1955, 1958) investigated the leakage of a number of substances from tissues into the surrounding medium following the action of various agents. He studied in greater detail the leakage of creatine from muscles and testes. The experiments showed that following the action of temperature, mechanical, chemical, and radiation stimulants, metabolic poisons, and asphyxia corresponding to the strength of stimulation, a greater or a lesser amount of creatine leaked out from the tissues into the surrounding solution. Together with other signs of excitation (increased affinity to dyes, etc.) the leakage is a sensitive index and a measure of the change in protoplasm.

Thus, the few papers available so far on the biochemistry of local excitation confirm the assumption that at the site of application the various stimulants cause, on the one hand, a nonspecific, repetitive complex of
84 visible changes in the living protein substrate (paranecrosis), and, on the other hand, serve as a stimulus to a slightly specific, catabolic, and glycolytic phosphorus metabolism. It is assumed that one is closely linked with the other, that paranecrotic changes in the protein substrate are primary, and that they serve as a stimulus for the biochemical reaction. In the chapter dealing with cell permeability, evidence will be brought forward to substantiate this hypothesis.

Certain Features of Paranecrosis during Physiological Stimulation of Cells

So far, the reaction of protoplasm to the stimulants at the site of their application has been discussed. In the majority of cases these stimulants were nonphysiological. Among the reactions were the biphasic change in viscosity of the protoplasm, decrease in the degree of its dispersion, and increase of its ability to bind basic and acid dyes. The latter was one of the most characteristic and interesting properties, since it indicated deep changes in the structure of protein molecules composing living protoplasm. A number of facts supported the contention that at the basis of these changes were reactions similar to the initial phases of denaturation of native proteins. This local reaction of protoplasm at the site of application of the stimulant was considered as local stable stimulation. This point of view is by no means generally accepted and requires special discussion. The point is that "stimulation", in the narrow sense of this word, usually indicates an active condition, mainly of the conducting tissues—nerve, muscle, and partly glandular epithelium. Very little is known as yet regarding other epithelial elements. Therefore, to show that paranecrosis may indeed be regarded as local stimulation, it must be ascertained whether the components of the conducting tissues themselves undergo paranecrosis during stimulation.

The difficulty in this type of comparison is that excitation in conducting tissues is rhythmic, the excitation waves being rapidly replaced by restoration of the initial, nonstimulated condition. This restoration may be incomplete, and in that case residual changes will remain. Or, the process of restoration may be overdone and the residual changes will then be totally different from excitation. It is known that changes of this kind are observed after passage of a wave of excitation, in the form of increased excitability (exaltation or supernormal phase).

Table 18

Change in binding of dyes by living protoplasm during physiological stimulation

Object ①	Stimulation ②	Dye ③	Dye concentration, % ④	Change in sorption (%) or result of microscopic examination ⑤	Author ⑥
Nerves of Anodonta cygnea (the cerebro-visceral connective).	Separate shocks by rectangular pulse	Neutral red Methylene blue Cyanol	0.1 0.1 0.5	+36.4± 8.3 +36.8± 13.0 +54.3± 10.0	Golovina, 1949, 1955/6.
Crab nerves	Induction current, 88 cps	Neutral red	0.005	+15.5± 3.4	Ushakov, 1950.
Frog nerves	Induction current	" "	0.02	0	Smitten, 1949.
	Sinusoidal alternating current, 100 cps	" "	0.2	+58.0± 7.2	Nasonov and Suzdal'skaya, 1957.
Rat nerves	Rectangular pulses, 50 cps	" "	0.1	+19.2± 4.9	Rozental', 1958.
Parasympathetic ganglia of frog heart	Induction current through the nerve	" "	0.05	Diffuse staining	Kotlyarevskaya and Boldyrev, 1939.
Sympathetic ganglia of cat and rabbit	The same	" "	0.1	+48.3±16.0	Zarakovskii and Levin, 1953.
Spinal ganglia of frog	" "	" "	0.005	+29.7	Ushakov, 1949.
	" "	" "	0.02	+56.7	Smitten, 1949.
	Rectangular pulses, 100 cps, through the nerve	" "	0.1	−29.6± 5.8	Lev and Rozental', 1958.
Spinal ganglia of rat	The same	" "	0.1	+21.6± 5.8	Rozental', 1958.
	Conditioned reflex stimulation	" "	0.1	+20.5± 5.5	Romanov, 1953b.

①	②
	Through the nerve, mechanical trauma of the limb
Spinal ganglia of rabbit	Through the nerve, induction current
	The same
Spinal ganglia of cat and rabbit	Through the nerve, induction current
Brain cortex of white mouse	Stimulation of mechano-receptors of the stomach and intestines of the esophagus
	Stimulation of the sciatic nerve by induction current
Brain cortex of white rat	Conditioned reflex stimulation
Motor nerve endings in frog muscle	Through the nerve, induction current

③	④	⑤	⑥
Neutral red	0.01	+20.0	Romanov, 1949b.
" "	0.1	+40.0	Romanov, 1948a.
" "	0.05	+36.7± 3.2	Romanov, 1948b.
Cyanol	1.0	+59.9± 8.2	
Neutral red	1.0	+46.3±11.0	Zarakovskii and Levin, 1953.
" "	0.05	+64.0	Levin, 1952.
" "	0.05	+46.0	
Phenol red	0.04	+23.5	
Neutral red	0.1	+11.7± 1.8	Romanov, 1953a.
" "	0.1	+35.9± 7.1	Romanov, 1953b.
Methylene blue	0.02	Increase in number of stained "tassels" by 162.2± 28.3%	Shapiro, 1953.
" "	2.0	Prevalence of stained endings in the experiment	Chetverikov, 1953.

①	②	
Sartorius frog muscle	Direct stimulation by induction current	
	Through the nerve, induction current, 25 cps	
	The same, 150 cps	
Straight abdominal muscle of the frog	Acetylcholine 1:25,000	
	Through the IVth, Vth, VIth spinal nerves, induction current	
Iliofibular frog muscle	Through the IXth root (tonus), induction current	
	Through the VIIIth root (tetanus), induction current	
Denervated rat muscles	Acetylcholine	
Embryonal rat muscles	"	
Mouse pancreas	Reflex	
Epithelium of rat cornea	Reflex stimulation. Repercussion changes.	12 hours after burning the twin eye.
		On the second day after cutting the sciatic nerve.

③	④	⑤	⑥
Neutral red	0.2	+21.9± 5.4	Kiro, 1948.
Cyanol	1.0	− 2.9± 6.0	
Indigo carmine	1.0	+ 1.3± 4.0	
Neutral red	0.05	+21.1± 5.6	Shapiro, 1958.
" "	0.05	+22.9± 3.1	
" "	0.2	+51.4±12.0	Kiro, 1948.
Cyanol	0.5	− 9.0± 2.7	
Neutral red	0.2	+14.0± 1.0	Vereshchagin, 1949.
" "	0.2	+15.0± 2.0	
" "	0.2	+ 8.7± 1.7	
" "	0.2	+150.0	Genni, 1947.
" "	0.2	+120.0	
" "	0.1	+20.9± 3.7	Nasonov and Suzdal'skaya, 1953.
" "	0.01	+30.0	Zhinkin and Korsakova, 1951.
" "	0.01	On the operated side, +30.0±2.2, on the opposite side, +55.0±2.7	Zhirmunskii, 1955.

Since vital staining is a comparatively slow process, it could be anticipated that these residual changes could be detected while studying alterations in binding power.

Recently, in our laboratory as well as in others, many studies of this type have been performed, and much data accumulated (Table 18).

Table 18 shows an increase in the sorption properties of dyes by protoplasm following stimulation. These were noted by different authors in nonmedullated and medullated nerves; nerve cells of spinal, sympathetic and parasympathetic ganglia; cerebral cortex; motor endings of nerves; skeletal muscles; the pancreas, and corneal epithelium. The greatest increase in staining occurred after acetycholine stimulation of muscles, which, since this stimulus is apparently nonoscillating, is logical.

Increase in staining power of stimulated nerves was observed for the first time in the cerebro-visceral connections of fresh-water mussels (Anodonta cygnea), (Golovina, 1949, 1955b), and in crab nerve (Ushakov, 1950). Conduction in the former is characteristically slow, with a prolonged restoration phase (Fick, 1863, Zhukov, 1936a, 1936b, 1946; Kan and Kuznetsov, 1938; Zhirmunskaya, 1940; Zhukov and Streltsova, 1943). These conditions facilitate the detection of increased binding of the dye. Table 18 shows increased staining following stimulation of the nerve with basic dyes (neutral red and methylene blue), and an acid dye—cyanol. This shows that in the given case, the increase cannot be explained either by shift in intracellular pH or by release of nucleic acids. Zhukov (1946), tested the conduction of an impulse in the nerve of fresh-water mussel (Anodonta cygnea). On increasing the distance between the stimulating and recording electrodes, a decrease in the amplitude of the action potential was seen. This may have been due either to increase in dispersion, or to decrease in the area of the summation curve of action current. Golovina tested the staining power of the nerve of fresh-water mussel (Anodonta cygnea), in the vicinity of the stimulating electrodes (0.5 cm) and at a certain distance from them (1.5 cm and more). Table 19 shows the results of staining ten pairs of such nerves, the staining of the proximal sector being always taken as 100%.

89 Table 19 shows that at a distance of 1.5 cm from the electrode, the nerve sector binds neutral red by an average of 14% less than the proximal sector. Further from the electrodes, this difference increases even more (experiments no. 8-10), in certain cases more than 40%.

Ushakov (1950) studied increased staining following stimulation of another nonmedullated nerve (i.e.of crab). This author used induction shocks from a DuBois-Reymond coil, in the primary circuit of which a Bernstein breaker, calibrated on an oscillograph, was introduced. The crab nerve is physiologically similar in many respects to vertebrate nerve, but differs in longer duration of the restoration processes. A significant increase in staining power ($M = +15.5 \pm 3.4\%$) was obtained only at a frequency of 88 cps (Figure 50).

At first, no increase in staining power of a stimulated medullated nerve could be observed (Smitten, 1949). This was explained either by the presence of myelin, interfering with penetration of the dye, or by the fact that in medullated nerve, complete restoration of the properties of the resting nerve fiber occurred rapidly. Subsequently it transpired that the failure to detect the increase in staining was due to the fact that upon stimulation with induction current, the frequency dosage for excitation was not

precise (the increase in staining depended to a great extent on the frequencies of the impulses, as following experiments clearly showed). The sciatic nerve of the frog was excited by superthreshold stimuli of sinusoidal current* from a sound generator, and simultaneously stained with 0.1% neutral red (in Ringer's solution) for 15 minutes. Figure 51 shows the results. Each point represents a statistically significant mean of 10 experiments (Nasonov and Suzdal'skaya, 1957). An optimum increase in sorption
90 is seen, reaching 58% at 100 cps. However, this result was obtained only during winter months. With summer frogs, the percentage of increase in staining was lower.

Table 19

Decrease in staining power of the nerve of fresh-water mussel (Anodonta cygnea) by 0.1% neutral red following stimulation (according to Golovina, 1955b)

Experiment No.	Distance from electrodes in cm	Staining (in % of staining of the nerve sector closest to the electrodes)	Difference in staining (%)
1	0.5	100.0	−15.2
	1.5	84.8	
2	0.5	100.0	+ 1.4
	1.5	101.4	
3	0.5	100.0	−13.0
	1.5	87.0	
4	0.5	100.0	−21.7
	1.5	78.3	
5	0.5	100.0	− 4.0
	1.5	96.0	
6	0.5	100.0	−36.0
	1.5	64.0	
7	0.5	100.0	−10.4
	1.5	89.6	
Arithmetic mean			−14.1 ± 4.6
8	0.5	100.0	−19.9
	3.0	80.1	
9	0.5	100.0	−10.4
	1.5	89.6	−34.2
	3.5	65.8	
10	0.5	100.0	−44.0
	3.5	56.0	

---

* The thresholds were determined by contraction of the calf muscle connected to the nerve.

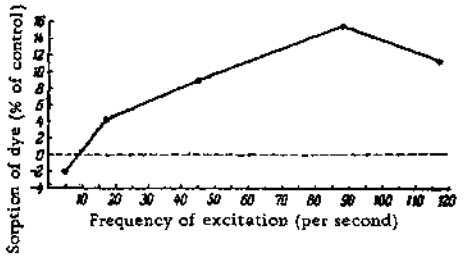


FIGURE 50. Sorption of neutral red by crab nerve following stimulation with induction current at different frequencies (according to Ushakov, 1950)

There are indications that in stimulated nerves, paranecrosis is observed in addition to the above changes. Thus, Kornakova, Frank, and Steinhaus (1947) observed a slow increase in viscosity of the sciatic nerve of frog, following a tetanic stimulus. Flaig (1947) observed a decreased rate of outflow of axoplasm from the cut end of the giant axon of the cuttlefish following its excitation. This undoubtedly indicated an increase in viscosity of its protoplasm. Later, Lyudkovskaya (1952) described a sharp decrease in transparency of the fresh-water mussel (Anodonta cygnea) nerve after stimulation, indicating a decrease in the degree of protoplasm colloid* dispersion.

Finally, in an interesting paper by Ungar, Aschheim, Psychoyos and Romano (1957) it was shown that in proteins excreted from stimulated frog and rat nerves, and proteins, the stimulated cerebral cortex of cats, the same shifts in the ultraviolet absorption spectrum occurred as in denaturated proteins**.

Another group of experiments on the increase in sorption of dyes by stimulated protoplasm was carried out on nerve cells of spinal ganglia of frogs and mammals. These nerve cells have an unusual structure in that they connect with the nerve fiber by a lateral (T-shaped) appendage, and that impulses may therefore pass in the direction of the spinal cord even when the ganglia are completely excised. Thus, there are no direct indications of impulses entering the ganglia and reaching the nerve cells.

---

* Contrary to this, Lyudkovskaya and Frank (1952) described increase in transparency in the medullated frog nerve after excitation. In certain cases, however, increased opacity of the stimulated nerve was also observed.

** Thus, the authors confirmed the results published in our monograph "Denaturatsionnayateoriya povrezhdeniya i razdrazheniya" (Denaturation Theory of Damage and Excitation), Nasonov and Aleksandrov, 1940). The basic assumptions of this theory were published in German ("Acta Zoologica", Nasonov and Aleksandrov, 1943b) and later in French, ("Transactions of the 13th International Congress of Zoologists in Paris", Nasonov, 1949b). Evidently, the authors were not familiar with all these papers, since they refer only to our paper published in "Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya (News of the Academy of Sciences of the USSR, Biological Series), in 1948, in which, by the way, denaturation of protoplasm proteins was not mentioned. On this basis, the authors state that we furnished no experimental proof of the theory of excitation, and that they are supposedly the first to have done so.

91

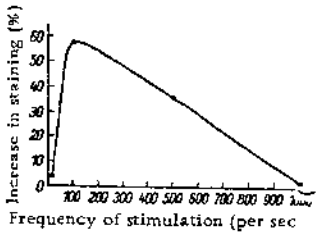


FIGURE 51. Increase in sorption of neutral red by the sciatic nerve of frog, after excitation by sinusoidal current of different frequencies (according to Nasonov and Suzdal'skaya, 1957)

The reaction to stimulation of sensory nerve cells of spinal ganglia was first detected by Romanov (1949c) using vital staining in a rabbit given an experimental shock. Shock was induced by the method of Cannon, i.e. smashing the muscles of the rabbit leg, using a hammer. Increased staining power of cells, as well as certain signs of paranecrosis, could be observed not only in ganglia directly connected with the stimulated fibers, but also in ganglia located higher. This indicated a spread of the stimulus along the nervous system of the animal. When the nerve was tied, or blocked by the use of novocaine, no shock was observed. Correspondingly, no increase in staining power was observed. On narcotising the animal, shock did not occur, and increase in staining was observed only in the cells of ganglia directly connected to the stimulated nerves. These experiments were performed on the whole animal, in which the blood circulation was preserved intact. Only the staining of nerve elements was performed on isolated ganglia.

Other experiments were performed with ganglia isolated together with the sciatic nerve. One end of such a nerve, with ganglia attached, was submerged in neutral red prepared in Ringer's solution. The other end was placed on the stimulating electrodes. The control preparation was simultaneously stained without stimulation. The experimental ganglia stained deeper than did the controls.

Ushakov (1949) performed similar experiments with frog ganglia. Since their dimensions are very small, the author had to place 20-40 ganglia in the same test tube in order to extract the dye with alcohol. On colorimetric analysis of such an extract, a result was obtained corresponding to an arithmetic mean of more than 20 experiments (Table 20). In order to determine the experimental error, Ushakov performed control experiments. These showed that the difference (in the amount of bound dye) between two series of twin ganglia (18 ganglia in each), stained with 0.005% neutral red under similar conditions, was ± 4.6%.

Table 20 shows that after stimulation for 40-60 minutes, the increase in staining of the stimulated ganglia, as compared with the control, may reach 30%.

Similar experiments were performed by Smitten (1949), who succeeded in increasing staining of the experimental ganglia by 56.7% more than the controls. It is interesting that Smitten obtained considerable increase not only by stimulation of the nerve end by electric shocks, but also by such stimulants as burns or cuts for 30 minutes, which completely excluded the possibility of excitation of the cells by current loops passing through the nerve.

However, increased staining of ganglia does not always occur after stimulation of the nerve. Lev and Rozental' (1958) stimulated the sciatic
92 nerve of a frog with rectangular impulses of 0.05 millisecond duration at a frequency of 100 cps. The sorption values of the experimental ganglia as compared with the controls differed widely in the various experimental series, an increase as well as a decrease in sorption being observed.

The mean sorption values, expressed as a percent of the control, were as follows: -20.9 ± 4.3; $+26.6 \pm 11.2$; $+16 \pm 7.5$; -11.8 ± 3.6; -0.3 ± 8.6; and -29.6 ± 5.8. It was observed that the result depended on the initial functional condition of the preparation, as shown by the degree of sorption of vital stain by the control ganglia.

Table 20

Vital staining of spinal ganglia of frog after stimulation of the nerve (according to Ushakov, 1949)

Experimental series	Number of ganglia	Strength of stimulation in cm	Intensification of staining of stimulated ganglia (%)
1st	24	25	123.0
2nd	47	20	129.7
3rd	22	15	113.6

On stimulation of rat nerves by rectangular impulses of the same frequency and duration, Rozental' (1958) showed that increased staining of the spinal ganglia always occurred, as compared with the controls. The mean sorption value from 21 experiments was $+21.6 \pm 5.8\%$. The level of sorption of dye by the control ganglia in the rat was very stable and varied from one series to another by not more than $\pm 10\%$. In the frog, these changes reached 200%.

Romanov (1948b) showed that after physiological stimulation of nerve cells of rabbit ganglia, increased sorption occurred of both basic dyes (neutral red) and acid dyes (cyanol). As in experiments with the nerve, this showed that increased staining power was not due to shifts in pH, release of nucleic acids, or lowered granule formation.

In another paper, Romanov (1948a) studied the changes in staining of nerve cells of spinal ganglia of the rabbit, at different periods after cessation of a 20-minute excitation through the nerve. Immediately after the impulses ceased, the staining of the excited ganglia was more than 40% greater than the control. Subsequently this decreased, reaching normal after 30 minutes of "rest". Forty minutes after cessation of the stimulus, staining power was 25% lower than the control. After 80 minutes, the staining returned to its initial level.

In addition to increased staining power, certain other signs of paranecrosis were observed in the protoplasm of excited nerve cells. Thus, Kotlyarevskaya and Boldyrev (1939) described an acid shift in the intracellular reaction of parasympathetic nerve cells of frog heart, while Stepanova and Krokhina (1941) observed an increase in glow in the dark field of these cells after stimulation of the vagi and sympathetic nerve fibers. Finally, Makarov (1948a), using preparations fixed in osmic acid, described the nuclear structures of sensory nerve cells of spinal ganglia of the frog after stimulation of the nerve. In nonstimulated cells, the nuclei in the osmic acid preparations were structureless.

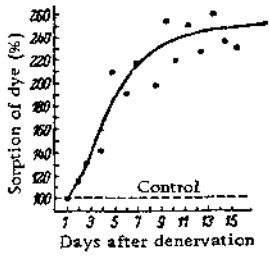


FIGURE 52. Sorption of neutral red by denervated rat muscle (expressed as a percentage of the amount of dye sorbed by 1 mg of normal muscle tissue from the opposite side). The muscles were stained in the presence of acetylcholine (according to Genni, 1947)

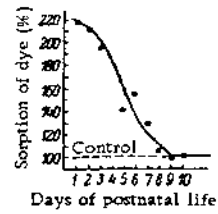


FIGURE 53. Effect of acetylcholine on sorption of neutral red by muscle of a newborn rabbit (as a percentage of the mean value of the amount sorbed under the same conditions by muscle of an adult animal) (according to Genni, 1947)

The following studies were performed on the cerebral cortex of mammals. Staining was carried out either after removal of the membranes from the isolated brain or on the whole organism by the method of Levin (1952). In the latter case, part of the skull and the cerebral meninges of a white mouse were removed under light narcosis. Under such conditions the animal may live for some time, retaining normal mobility and capacity to partake of food. Vital staining was performed after attaching the
93 brain to a thermostat and submerging the exposed surface of the brain to the dye, heated to body temperature. Microscopic examination of the living brain after such staining showed uniform diffusion staining of cells and fibers. After staining, a segment of a given area was excised by a special stamp, this section being submerged in acidified alcohol to extract the dye. The extract was measured colorimetrically in the usual way, and the intensity of staining of the "stimulated" brain was determined as a percentage of the "control" brain.

Levin (1952), in his experiments, excited the mechanoreceptors of the stomach and the esophagus, which gave a considerable increase in staining of the "stimulated" brain over that of the control. In the case of the basic dye (neutral red), this increase was 46-64%, and with an acid dye (phenol red), 23.5%.

Romanov (1953b), using white rats under the influence of a conditioned reflex stimulus, found an increase in staining power of the cerebral cortex and spinal ganglia. The rats were placed in a special cage, the base of which was a net of parallel metal rods. Alternating current of 30 volts was passed through the rods. Each time, approximately 2 seconds before the current was introduced, an electric lamp of 100 watt, mounted in the

cage, was switched on. As a rule, by the 8th-9th day, a defense reflex appeared in the rats. After being placed in the cage and after the lamp was lit, a strong stimulation appeared in the rats.

A day after such a serial combination, the rat was placed in the cage and subjected to the action of the environment, without reinforcement. The animal was then killed by decapitation. The brain and ganglia were immediately isolated for subsequent staining. The controls were rats which elaborated the defense conditioned reflex but which, on the day of the experiment, were not subjected to a nonconditioned or to a conditioned stimulation. The staining of the experimental brain exceeded that of the control by 35.9 ± 7.1% (average of 19 experiments), and the staining of the experimental spinal ganglia exceeded that of the control by 20.6 ± 5.5%.

Shapiro (1953) first observed that the staining power of motor nerve endings of the frog by methylene blue (0.02%) increased after stimulation. The experiments were performed as follows, using twin muscles (m. sartorius). One was stained after stimulation via the nerve, the control was stained without stimulation. The staining lasted 4 minutes, after which the muscles were rinsed in Ringer's solution and kept for 4-5 minutes on a glass slide with free access of air. Subsequently, the stained nerve endings were counted under the microscope in the experimental and control muscles. At a frequency of stimulation of 25 cps, the count in the experimental muscle exceeded the control by an average of 162.2% (m = ± 28). These experiments were repeated and confirmed by Chetverikov (1953) on m. ileofibularis of the frog.

Finally, there are studies demonstrating increased vital staining of skeletal muscles after stimulation. This was first shown by Kiro (1948), on direct stimulation of frog sartorius muscles with induction current.
94 The results showed a statistically significant increase in staining with neutral red—+21.9 ± 5.4%. It is interesting that in contrast to nerve cells, this increase was observed only with basic dyes. Acid dyes (cyanol and indigo-carmine) showed no increase.

After stimulating the straight abdominal muscle with acetylcholine (dilution of 1:25,000 in a solution of neutral red) an increased staining of 51.4 ± 12% was observed, while in an acid dye (0.5% cyanol) or on electric stimulation, no increase was observed. Evidently, after stimulation, the difference between the protoplasm of nerve elements and muscles consisted in the fact that in the latter, mainly negatively charged spots of protein molecules were activated.

Extremely interesting data on the increase in vital staining of muscles of mammals following the action of acetylcholine were produced by Genni (1947).

The capacity to be stimulated (i.e. to contract) in response to acetylcholine is characteristic of embryonic muscles. In the postnatal period, this property becomes daily weaker, disappearing altogether on approximately the 10th day, with a corresponding decrease in the ability to bind dye. However, on the 8th-9th day after denervation of the muscles, their ability to react to acetylcholine is restored with a simultaneous increase in staining power by neutral red. The latter fact is characteristic of protoplasmic excitation.

The work of Vereshchagin (1949) carried out in Zhukov's laboratory confirmed these observations. He studied the m. ileofibularis of the frog. This muscle consists of bundles of tetanic fibers innervated by the eighth

95 root, and tonic fibers innervated by the ninth root. After staining during stimulation of the latter, an increase in dye sorption of 15% (± 2) was seen. After stimulation of the former, sorption increased only by 8.7% (± 1.7). Thus, tonic contraction caused a sorption effect twice as strong as tetanic contraction. This result might have been expected a priori. Stimulation (via the nerve) of the straight abdominal muscle, consisting mainly of tonic elements, gave the same results.

Paranecrosis and Stimulation of Gland Cells

There is an extensive literature on the nature of muscle contractions and conduction of nerve impulse, but the mechanism of action of the secretory gland cells is as yet little known.

Almost nothing is known of the biochemical processes underlying secretion. Little is known about the mechanism of water filtration through the secretory cell, sometimes taking place against osmosis, with the secretion developing a pressure greater than that of blood. Likewise the mechanism by which secretory granules are deposited in the protoplasm of glandular elements is not known, nor how these granules dissolve rapidly after stimulation of the gland and are excreted into its lumen in the form of a liquid secretion.

These problems of secretion and filtration ought to be solved by predominantly biochemical and biophysical methods. The problems of accumulation and removal of the prosecretion granules can be solved by a combined method, using physiological experiments, study of fixed histological preparations, and in vivo microscopic observations. One example of this type of study is the old classical work from the laboratory of I.P. Pavlov, published by the physiologists Babkin and Savich, together with the histologist Rubashkin (1909).

A series of studies performed approximately 30 years ago showed that the secretion granules in the cells of various glands appear in the region of the so-called reticular apparatus of Golgi, from whence they spread to the whole cell (Nasonov, 1924, 1926). The Golgi apparatus is thus the excretory organ of the cell. It should however be noted that these studies were predominantly morphological, and the problem of the appearance of granules in cells was examined topographically. No attempts were made to examine the physicochemical aspect of the process. How the accumulated granules moved from the cells into the lumen of the gland was not dealt with at all.

It was stated at the beginning of this book that physiologists often use the term "excited cells" to describe the active condition of different tissues (i.e. muscle, nerve, and secretory cells). This is to emphasize that there is something in common between apparently different manifestations of physiological activity, such as mechanical work of muscles, con-
96 duction of an electronegative wave through the nerve fiber, and excretion of substances by gland cells.

One of the most important tasks of general physiology is to find the distinguishing properties in a "stimulated condition" of different tissues.

Data from the literature show that "stimulated" protoplasm binds more vital stain than resting protoplasm. This increase may be due to

activation of ionized groups in live protoplasm. It is assumed that these changes are similar to paranecrosis. If secreting gland cells really enter a state similar to excited nerve and muscle cells, then it is natural to assume that their protoplasm should change accordingly. In particular, an increased sorption of dyes might be expected.

To test this assumption, experiments were performed on vital staining of the pancreas in fully fed and in starved white mice. It was first necessary to establish the time periods of mild hunger when the pancreas cells were maximally loaded with prozymogen granules, since a too prolonged hunger may cause emptying of the gland. The staining was performed in the following manner: the mouse was decapitated, rapidly dissected, and the carefully isolated pancreas immersed for 30 minutes at room temperature in Ringer's solution (without sodium carbonate) containing 0.1% neutral red. A section of the gland was examined microscopically before staining, to check whether there were granules in the cells. After staining, the glands were rinsed in water and the dye extracted with acidified alcohol. The glands were then dried and weighed. The ratio of staining intensity to dry weight of the gland was determined, followed by the determination of the percentage ratio of staining of the experimental glands (from fed mice) to the controls (from starved mice).

In the 8 experimental series performed, glands from 40 fed mice and from 40 starved ones were examined (Table 21).

Table 21

Intensity of vital staining of mouse pancreas (according to Nasonov and Suzdal'skaya, 1953)

Dry weight of mice glands, mg		Ratio between vital staining intensity of glands from fed and from starved mice (%)
fed	starved	
66	78	122.1
72	80	116.5
45	57	112.2
53	53	112.35
40	50	122.9
31	33	139.1
26	42	132.8
36	34	118.9
Arithmetic mean		
46	53	122.1± 3.74

97 Table 21 shows that in all 8 series, glands of the fed mice (stimulated) bound the vital stain by an average of 22.1% more than glands from the

FIGURE 54. Effect of high hydrostatic pressure on cells of mouse pancreas (according to Nasonov and Suzdal'skaya, 1953)

A--paranecrotic cells of a mouse starved for 5 hours. Prozymogen granules and chondriosomes are seen; the nuclei are weakly stained and show weakly developed structures. B—cells of the same gland, subjected to a stimulating effect of 15% ethyl alcohol (in Ringer's solution) for 20 minutes before fixation. The prozymogen granules disappeared completely, the number of chondriosomes decreased, the nuclei were paranecrotic and somewhat smaller in volume, with well-marked structures. C—paranecrotic cells of a mouse starved for 5 hours. Prozymogen granules, threadlike chondriosomes, and nuclei with indistinct structures are seen. D-cells of the same gland, after hydrostatic pressure of 2,000 atmospheres for 10 minutes. The prozymogen granules disappeared completely, the nuclei showed distinct structures, the chondriosomes remained unchanged. Treatment: fixation according to Ciampi (strong mixture), mounted in paraffin, stained according to Kul'. The drawing device was at the level of the working table. Magnified: 90 x 20.

starved mice (nonstimulated glands). (Note: the dry weight of glands from starved mice is somewhat higher, on the average, than that of glands from fed animals, due to excretion of granules of pro-secretion from the cells, and removal of the secretion from the lumina. However, this could only reduce the obtained increase in staining, since the latter was related to a unit of dry weight; and the granules of secretion, as shown by in situ microscopic observation, are stained somewhat stronger than the protoplasm. Therefore removal of a stronger staining substance from the cell ought to decrease sorption of the dye).

In the experimental and control glands, after 30 minutes of staining with 0.1% neutral red, granules of the stain could be seen on microscopy. These granules were relatively scarce, and the main part of dye binding was attributed to diffusion staining of the protoplasm, thus accounting for the increase in staining of stimulated ("fed") glands, as compared to resting ("starved") ones.

Thus, the excited glandular cell of the pancreas is similar in one respect to stimulated muscle and nerve elements: it binds more dye than cells in the resting state. Since following the action of stimulants, an increase in sorption of dye in general is characteristic of all the cells of various animal and plant organisms, the question arose whether it was possible to cause experimentally the dissolution and disappearance of granules from glandular cells in an isolated gland, by the direct action of nonspecific stimulants. In other words, whether or not it was possible to make the gland react by something similar to secretory activity, in the same way as different stimulants may cause contraction of muscles or parabiosis in nerve conductors. In order to solve this problem, the following series of experiments was performed.

Mice, in a condition of mild hunger (i.e. with pancreatic cells filled with prozymogen granules), were decapitated, and the pancreas excised. One section of the gland was examined in vitro under the microscope, while another was fixed according to Ciampi. The remaining part of the pancreas was used for experiments with different stimulants. Different time intervals and different doses were used, after which a further section was removed for control and another for fixation.

The effect of the following chemical and physical stimulants was investigated: ethyl alcohol, isoamyl alcohol, ethyl ether, potassium choride, calcium chloride, acetic acid, hydrochloric acid, sodium hydroxide*, hy-
98 pertonic sodium chloride in Ringer's solution, elevated temperature and high hydrostatic pressure, maintaining constancy of the gas composition. The majority of these agents, in certain concentrations, caused complete evacuation of prozymogen granules from the gland cells (Table 22 and Figure 54).

The fourth column of Table 22 shows the concentrations, or the intensities, of the agents causing contraction of skeletal muscles and noticeable paranecrotic changes in other tissues after 1 hour.

In most cases the doses are similar to those causing emptying of granules from glandular cells (in vitro). Potassium chloride, calcium chloride and sodium chloride (hypo- and hypertonic) are exceptions. The first two cause emptying only at very high concentrations as compared with the paranecrotic ones, while sodium chloride causes no emptying after any

---

* All the above-mentioned substances were added to Ringer's solution for warm-blooded animals.

changes of its normal concentration in Ringer's solution. It is highly probable that the reason for this is, that with natural secretion, the salt concentration of the secretion may change within a very wide range, and the glandular cells may be well adapted to this factor.

Table 22

Doses of different agents necessary for removal of secretion granules from pancreatic glandular cells (according to Nasonov and Suzdal'skaya, 1953)

Agent	Concentration or intensity	Time of action, min	Threshold concentration or intensity of the agent, necessary to cause contraction of muscles or paranecrosis of other tissues, within one hour
Ethyl alcohol	15-20%	30-45	16.0%
Isoamyl alcohol	1%	45	0.5%
Ethyl ether	2.5%	100	5.0%
Potassium chloride	9.6%	155	0.2%
Calcium chloride	8%	145	1.5%
Acetic acid	0.2%	15-55	0.06%
Hydrochloric acid	0.01 N.	10	0.01 N.
Sodium hydroxide	0.03 N.	13	0.01 N.
Temperature	50° C	70	37-40° C
Hydrostatic pressure	1500-2000 atm.	30	--

Figure 54 A illustrates a group of glandular cells from a pancreas of a moderately starved mouse. Considerable accumulation of prozymogen granules at the alveolar lumen of each cell is seen. The threadlike and granular chondriosomes typical of the pancreas are orientated along the axis
99 of the cell. The nuclei are hardly noticeable and almost entirely structureless, characteristic of preparations fixed with good fixatives containing osmic acids. As shown by Makarov (1948a, 1948b), these fixatives clearly reflect the condition of the normal nuclei.

Figure 54 B illustrates a preparation of the same gland, after 20 minutes in 15% alcohol (in Ringer's solution). Here, the glandular cells are completely free of prozymogen granules. The chondriosomes are preserved, but are more granular. The nuclei have a typical paranecrotic appearance. They show well-defined structures, somewhat wrinkled, and their chromatin stains intensely. Makarov (1948a, 1948b), using many tissues, convincingly showed that osmic fixatives preserved the differences existing in vivo between normal and paranecrotic cells. These differences are beautifully shown in Figure 54 B. There can be no doubt whatsoever that the cells are in a condition of marked paranecrosis, clearly seen on examination in situ.

Figure 54 C shows an acinus of a normal pancreas. As in Figure 54 A, a considerable accumulation of secretion granules is seen, and markedly

elongated, threadlike chondriosomes with very indistinct structures, and almost unstained nuclei. Figure 54 D shows the same gland, after 10 minutes of a hydrostatic pressure of 2,000 atmospheres (the content of the gases dissolved in Ringer's solution did not change during the experiment). Although in this case the nature of the stimulus was quite different, the results were similar in general to those obtained in the previous experiment in which alcohol was used. However, one difference was that the chondriosomes did not lose their threadlike appearance and the protoplasm was better preserved.

In order to show that under the pressure used the pancreas really became paranecrotic, the gland was placed in Ringer's solution to which 0.1% neutral red was added, and subjected to pressures of varying force. The stain was extracted with acid alcohol as described above, and measured colorimetrically. Table 23 shows the experimental results.

Table 23

Increase in vital staining of mouse pancreas under high hydrostatic pressure (according to Nasonov and Suzdal'skaya, 1953)

Pressure, atm.	Increase in staining of experimental glands in relation to the control ones (%)
1000	+35.2
2000	+57.4

Remark: The staining was related to dry weight.

Control experiments to establish the degree of staining of fixed tissues have shown that pressure exerts no effect on staining of dead substrate.

Thus, after natural excretion from pancreas cells the latter show increased sorption to dyes. This is also characteristic of other cells subjected to stimulation.

Consequently, these experiments demonstrate true paranecrosis, or parabiosis in its widest sense.

The question however arises whether paranecrosis can, in some way, cause the removal of secretion granules from the cells.

At the moment it is only possible to make observations on deposition of vital stains and to draw a few conclusions. Vital stains are deposited in cells in the form of granules. This process resembles the deposition of secretion granules insofar as both granules are deposited in the loops of the Golgi apparatus. It is also known that in paranecrosis the cells lose their ability to form granules, and the dye diffusely stains both protoplasm and nucleus. In this connection, a new question arises: whether the dye granules can return to a soluble state under the influence of paranecrosis. If this were so, some information on the process of excretion of granules from the cell might be obtained.

A study of this sort was performed in our laboratory by Shtutina (1939). This author caused deposition of neutral red granules in cells of frog intestines and in the salivary glands of larvae of the Chyronomus mosquito. Subsequently, after various time intervals, she treated these cells with paranecrotic agents (hypotonicity, acid, asphyxia). The results showed that to a certain degree, the process of granule formation of the dye was reversible if the granules appeared in the cell relatively early. Dye granules not older than several hours easily returned to the diffuse state under the influence of any paranecrotic agent tested. Older granules (24 hours

and more) did not disappear even as a result of the action of the strongest agent. The reason for this behavior, according to Shtutina, was that as pointed out by Khlopin (1927), the dye granules in the cell showed certain changes with time, namely: a certain scarcely soluble protein substrate, which Khlopin called crinoma, combined with these granules. Younger granules of dye not yet bound to the crinoma could be removed from the cells. They served as a good physiological model of excretion of secretion granules by excited glandular cells which were possibly in the initial stage of paranecrosis.

Further studies in this direction will no doubt show what changes associated with paranecrosis of the cell cause dissolution of the secretion granules already formed.

This ends the review of the literature on changes in cell protoplasm after physiological stimulation. In our opinion this review justifies the assumption that certain identical or very similar paranecrotic changes take place in the protoplasm not only after stimulation by inadequate stimulants such as high temperature, mechanical factors, hydrostatic pressure, sound, radiant energy and chemical agents, but also after physiological stimulants (e.g. acetylcholine, or impulses along nerve or muscle fibers.
101 The above facts support our thesis that these changes resemble reversible denaturation changes in the proteins of living protoplasm.

Decrease in Vital Staining of Protoplasm

So far, only an increase in vital staining of living cells under the influence of various agents has been discussed. In many cases however, a decrease has been described. These observations are of special interest and require some discussion.

First of all, decreased vital staining is observed in cases of reversible change in protoplasm. If, under the influence of a stimulus the condition of an organism changes, thereby causing increased sorption of the dye, removal of this agent will lead to decrease in vital staining. This process may be considered as restoration of the living system after excitation. Many observations of this kind indicate that there are different levels in the state of live protoplasm, with very gradual transitions between them. These levels are usually called "physiological condition", a very loose term. The important point in this case is that the level of the physiological status of the protoplasm may change smoothly from a higher to a lower one and vice versa. The degree of vital staining, together with other indexes, e.g. the value of electronegativity, etc. may serve as a guide to this level.

Figure 55 illustrates the staining power of spinal ganglia of rabbits, after excitation through the nerve for 20 minutes. Immediately after cessation of the stimulus, staining of the ganglia increased by 40%. Later, this decreased, reaching the initial level (100%) after 35 minutes. However, the decrease in staining power did not cease; it continued up to 40 minutes, when the value of staining was 75% of normal. Later, it again increased slowly, reaching the initial intensity 80 minutes after cessation of the

stimulus*. This peculiar hyper-repair of protoplasm following the action of a stimulant was later described by Romanov in a number of objects: brain of mouse after a strong stimulation by electric excitation of the sciatic nerve; spinal and sympathetic ganglia of the rabbit after prior stimulation by strong sounds of an explosive nature (1954) and by ethyl alcohol (1949b), and frog muscles stimulated by high temperature (1949a). It is interesting that according to the data of Romanov (1953a) and others, protoplasm, after recovery from stimulation, possesses a lower staining power as compared with the initial level (i.e., increased nativity), and acquires a higher re-
102 sistance to various harmful agents (e.g. strychnine). According to Golovina (1958a) this condition is also characterized by increased excitability.

Thus, the staining power of protoplasm can be lowered by removal of the stimulating factor, with restoration of the status quo, as well as by other methods. In a number of studies, this was observed during the action of weak doses of various agents, which, on further increase, led to greater staining power of the protoplasm.

There is thus a biphasic effect. A good example of this is the change in vital staining of protoplasm following increase of hydrostatic pressure, obtained by Golovina (1955a). Figures 20 and 21 show that with increase of pressure to 200 atmospheres, staining of muscles decreased by 22%, while on further increase of pressure, binding of the dye increased, reaching +60% at 1,000 atmospheres. These data are of special interest, since they were obtained by the use of basic as well as acid dyes, excluding the possible effect of a pH shift.

A similar biphasic behavior of vital staining was observed by Zarakovskii and Levin (1953) who studied the change in sorption properties of cells of spinal and sympathetic ganglia of mammals after stimulation of the nerve by induction current. Figure 56 shows that a weak subthreshold stimulation caused a decrease in staining of the ganglia cells by more than 30%, while stronger stimulation caused an increase of almost 50%**.

In experiments performed by Zhirmunskii (1954, 1958) with denervated leg muscles of a rat, a similar biphasic nature of vital staining was found (decrease of 61% two days after cutting the sciatic nerve, and increase of
103 102% thirty days afterwards). These changes were compared by the author with the development of parabiosis of the denervated muscles.

Evidently, the phase of decrease in staining power is connected with increased excitability as described by Vvedenskii (1901); increase in resistance to harmful agents (Cherepanova and Suzdal'skaya (1954***); and with the capacity of longer survival outside the organism (Kiro, 1954).

---

* Romanov (1948a) explained the decrease in staining power after stimulation by the toxic effect of a strong dye solution, stronger for the control than for the experimental object. This assumption, however, has not been confirmed (Zarakovskii and Levin, 1953).

** The authors explain the effect of strong and weak excitation upon spreading stimulation by the gradual decrease in spreading of the weak subthreshold impulses, which could be observed with electrodes located near the ganglia.

*** The authors have shown on the basis of extensive data that a combination of two harmful agents, among which at least one is used in a small (subthreshold) dose, does not lead to summation of their harmful effects, but to their decrease. Evidently this explains many facts connected with the antagonistic action of the agents.

This phase of decrease in staining of the protoplasm, as compared to the normal, is also observed after the cessation of action of a strong stimulus. In this respect it has been shown by Golovina, 1958a, that an increase in excitability occurs, and (by Romanov, 1953a) that an increase in resistance to harmful agents also occurs.

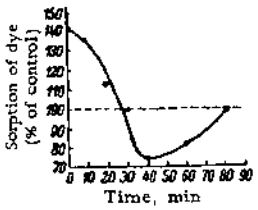


FIGURE 55. Vital staining of spinal ganglia of the rabbit with 0.1% neutral red, at different times after cessation of the stimulus (according to Romanov, 1948a)

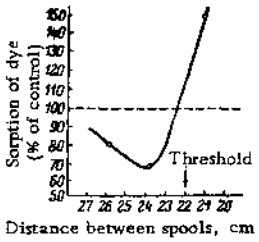


FIGURE 56. Binding of 0.1% neutral red by the upper sympathetic ganglia of the neck of a cat, during a 30-minute stimulation with induction current at a frequency of 25 cps, (according to Zarakovskii and Levin, 1953)

The increase in excitability of the nerve in the presence of small doses of agents which subsequently lead to suppression of excitability, was called by Vvedenskii (1901) "the prodromic stage of parabiosis". This author was the first to describe the increase in nerve excitability above the initial level after passage of a stimulation wave*.

The question arises: how can this multiphasic change be explained, first in the living substrate, and second in the physiological condition of cells after the action of stimulants. This question is of exceptional interest, since it deals with a widespread phenomenon. So far, too little data is at our disposal to justify a firm opinion. Only certain preliminary facts can be considered.

It is assumed that after the action of stimulants, changes in protoplasm proteins take place, similar to the initial stages of denaturation. These changes should stimulate biochemical metabolic processes directed towards their repair. However, there is no basis for the assumption that these processes will always necessarily restore the organism to its initial condition. It is highly probable that following excitation these restorative processes lead to renaturation of altered proteins, the level of which does not reach that which existed before their action, or exceeds it. It is possible that this increase above normal limits occurs in the initial period of action of a weak stimulant.

Something similar may also be assumed after cessation of a strong stimulus. A peculiar hypernativation is then obtained, accompanied by an increase of all the physiological indexes connected with it, e.g. excitability, resistance, etc.

It is emphasized that the above explanation is far from being definitive.

---

* This stage was called by Vvedenskii the stage of "exaltation", for which the term "supernormal phase" was later suggested by British physiologists.

Part II

EXCITATION AND PERMEABILITY

Chapter 1. The Membrane Theory of Cell Permeability and its Critical Appraisal

Cell Permeability and the Theory of Excitation

The study of the nature of cell excitation is closely associated with the problem of cellular permeability. Following stimulation of cells, characteristic changes occur, with considerable increase in permeability toward all substances entering the cell, as well as to substances excreted by them. Thus, the theoretical considerations which at the end of the last century formed the basis of the theory of cellular permeability, also formed the basis for the theory of excitation.

The postulation of the so-called membrane theory of permeability led to the membrane theory of excitation. It follows that any attempt to revise the former should inevitably lead to revision of the latter, which is now firmly established in physiology.

For many years we and our co-workers studied vital staining of tissues and cellular permeability. On the basis of our data and on the basis of analysis of papers published by other authors, we concluded that this membrane theory presents a false concept of cellular structure and of the condition of many substances contained in the protoplasm. At the same time the membrane theory, due to its apparent simplicity, and its application in the explanation of various baffling phenomena, acquired great popularity among physiologists, but led to many ill-directed researches.

The appearance of our papers on permeability caused some controversy*. That is why in the present book devoted to the problem of excitation, our concept of permeability will be stated in detail. This task is made considerably easier by the appearance of Troshin's basic book "The Problem of Cellular Permeability" (1956). In it the literature on this problem was collated and the membrane theory subjected to criticism. The ideas de-
105 veloped by us for many years—which we called "The Sorption Theory of Permeability", are also presented. Thanks to this, discussion can be limited here to basic principles.

---

* See collection: "Problema pronitsaemosti". Trudy Konferentsii Moskovskogo obshchestva fiziologov ("The Problem of Permeability". Transactions of the Conference of the Moscow Society of Physiologists, 1939); the book by Nasonov and Aleksandrov (1940) and papers by Nasonov and Aleksandrov (1943a, 1944).

Origin and Development of the Membrane Theory of Permeability

The theory that the protoplasm of cells is surrounded by a very thin membrane which is insoluble in water was expressed for the first time by Schultze (1863) and Kuhne (1864). According to these authors, the protoplasm should be regarded as a simple aqueous solution of organic (mainly protein) and mineral substances. However, the immiscibility of protoplasm with the surrounding water required a special explanation, and therefore Kuhne's hypothesis envisaged a membrane of coagulated proteins, while Shultze described a specially dense layer of protoplasm interfering with its solution in the surrounding water

Later, Pfeffer (1877) and de Vries (1884, 1885, 1888) observed that plant cells possessing a central vacuole behave like osmometers when placed in hypertonic solutions of substances which do not permeate the vacuole of the cell. These authors therefore assumed the presence of a very thin membrane on the outer surface of cells, permeable to water but impermeable to certain substances in solution. Pfeffer compared these membranes with the sedimentary membranes of Traube. He ascribed them an important role in the process of metabolism.

Thus, the idea of semipermeable membranes on the surface of cells arose during attempts to explain two properties of protoplasm described by cytologists: (1) immiscibility of protoplasm with water, and (2) reversible reduction of the cell volume in concentrated solutions of substances which probably do not permeate into the cell.

De Vries and other authors used this property to determine the degree of penetration of various substances into the cell. Thus, the first method for the study of cellular permeability was suggested, i.e. the osmotic method.

Later Overton (1895, 1896, 1902) observed that not only vacuolated plant cells change their volume in hypertonic and hypotonic solutions of impermeable substances, but that animal cells also have this property. Thus the hypothesis of semipermeable cell membranes also applied to animal cells.

Overton, and later also other investigators, used the osmotic method to study permeation of various substances into plant and animal cells. On the basis of many observations Overton determined a sequential series of substances according to their capacity to penetrate the cell and he noted certain general rules to be adhered to. This series was as follows:

1) Hydrocarbons (saturated, unsaturated and cyclic) and their haloid derivatives; monobasic alcohols; aldehydes; ketones; nitrites; ethers; many organic acids and alkalis. All penetrate rapidly into the cells.

)6 2) Dibasic alcohols and amides of monobasic acids permeate rapidly.

3) Glycerol, urea, thiourea and others permeate less rapidly.

4) Tetrabasic alcohols permeate slowly.

5) Hexabasic alcohols; sugars (pentoses, hexoses, disaccharides); various aminoacids; many neutral salts of organic acids; strong mineral acids, as well as salts of strong mineral acids and alkalis; all permeate very slowly or not at all.

Analyzing the data obtained, Overton reached the conclusion that compounds freely soluble in fat-like, lipoid substances penetrate the cells with greater ease. On this basis he was the first to propose the lipoid

theory of permeability, based on the observations of Quincke (1898) that each living cell has a lipid membrane on its surface, due to which its protoplasm is immiscible with water.

Overton's series is a paradox from the physiological point of view. According to his theory unnecessary or even toxic substances such as alcohol, ethers, aldehydes, ketones and others permeate very freely into the cell, while vitally important substances—without which the cell could not exist—sugars, aminoacids and mineral salts—either do not permeate or permeate very little. This fact is an enigma to many investigators. It even caused Höber (1926) to suggest a special theory of "physiological permeability", according to which permeability studied by ordinary rough methods elucidates only the physical properties of the membrane. In addition, according to Höber, some unknown mechanisms exist for the permeation of substances across the cell membrane, causing physiological permeability. But this theory simply substitutes one unknown for another.

The contradiction in Overton's series is due to an incorrect and indirect method used to study permeability (i.e. the "osmotic effect"). Actually, all the substances listed at the end of his series penetrate the cell at about the same rate. The difference is only in the equilibrium level of concentration within the cell.

In the course of its development, the membrane theory was often criticised with varying severity. One of the first critics was Fischer and his co-workers (Fischer and Moore, 1907; Roaf and Alderson, 1907; Moore and Roaf, 1908, 1913; Moore, Roaf and Webster, 1912; Fischer and Suer, 1935, 1938, 1939), and also Lepeshkina (1924, 1928, 1930, 1936). But to date the membrane theory of permeability is considered pre-eminent in contemporary physiology.

The Hypothetic Cell Membrane and its Suggested Chemical Composition and Structure

Cell protoplasm was thus considered as a simple aqueous solution, enclosed within a membrane permeable to water, but impermeable to many substances dissolved in it (sugars, salts, aminoacids, and others). This point of view is shared by almost all contemporary physiologists.

However, theories on the structure and chemical composition of the cellular membrane have constantly been modified according to newly-obtained but incompatible data.

107 Firstly it should be pointed out that direct proof of the existence of a semipermeable membrane on the surface of cells is still lacking. All the arguments in its favor are indirect. The existence of such a membrane is purely hypothetical. All those who recognize this agree that the membrane is not a morphological entity but a physiological one, and that it should not be confused with structures readily identifiable under the microscope, such as the membrane of the ovum, cuticular membranes of all kinds, and pellicles of epithelial cells or the sarcolemma of muscle fibers, etc. According to some authors, these formations are not endowed with semipermeability (Overton, 1902; Höber, 1926, 1945; Harvey and Danielli, 1939, and others). It is assumed that cell membranes are so thin that

they cannot be seen under the microscope. According to various fragments of indirect evidence, these membranes are thought to consist of several layers of molecules of a thickness of 30-200 A (Fricke, 1925; Danielli, 1936; Schmitt, Bear and Ponder, 1938; Fricke, Parker and Ponder, 1939; Dziemian, 1939, 1942 and others).

As already mentioned, Quincke (1898) assumed that the membrane is made of fat or oil, thus explaining the insolubility of protoplasm in water. Later, Overton, on the basis of studies on a permeability series, ascribed to the membrane a lipoid composition. However, while the latter may explain the permeation of certain substances, it does not explain the free entrance of water into the cell. In order to overcome these difficulties, Nathansohn's (1904a, 1904b) hypothesis assumed that the lipoid layer on the surface of the cell is not solid but porous. Through this layer, water and certain lipoid-insoluble substances dissolved in water may pass (the mosaic theory). This arbitrary modification of the hypothesis made it much more flexible and therefore more acceptable to physiologists.

Attempts to explain all permeability phenomena by porous filtration (the ultrafilter theory of Ruhland, 1908, 1909a, 1909b, 1911, 1912a, 1912b, 1913; Ruhland and Hoffman, 1925) have not gained recognition. The connection between the molecular volume and the ability to penetrate the cell only existed for certain substances (of very little surface activity), penetrating the protoplasm of the sulphur bactrium Beggiatoa mirabilis.

The lipoid-soluble, surface-active substances penetrate the cells very readily, notwithstanding the large dimensions of their molecules. The mosaic theory of Nathansohn explained the penetration of all substances, soluble and insoluble in lipoids. The former enter the cell, dissolving in the lipoid membrane, while the latter pass through the pores. Water, too, penetrates by passing through the pores. The selective permeability of ions remained unexplained. It was necessary to explain why certain cations penetrated the cell, while anions seemingly did not penetrate at all. An answer to this question was given by Michaelis (1925, 1926; Michaelis, Fujita and Dokan, 1925; Michaelis, Ellsworth and Weech, 1927; Michaelis and Perlzweig, 1927; Michaelis, Weech and Yamatori, 1927). He assumed that the walls of the cell membrane pores were negatively charged, as a result of which penetration of negatively charged anions was not possible. Michaelis illustrated his theory by experiments with artificial membranes. 108 Later the theory was elaborated on and perfected by Teorell (1935a, 1935b, 1936), and also by Meyer and Sievers (1936).

With these additions and elaborations, the membrane hypothesis is accepted by many investigators even now (Höber, 1936, 1945; Wilbrandt, 1938a, 1938b; Rubinshtein, 1947 and others).

But even in this form this hypothesis does not satisfactorily explain many facts. First of all, cell protoplasm contains potassium and phosphates at a considerably higher concentration than in the surrounding aqueous solution, while in the latter the concentration of sodium and chloride ions is considerably greater than that in protoplasm. At the same time, the membrane theory assumes that protoplasmic electrolytes all exist in a free dissolved state, because if this were not so, the cells could not function as osmometers. In the original variation of the membrane theory it was assumed that sodium, chloride ions and phosphates did not penetrate at all through the membrane pores, while potassium, although it penetrated,

could not leave the cell since it was bound to phosphates by electrostatic forces.

It is now known that such absolute impermeability does not exist. We know that upon stimulation and excitation of cells, potassium and phosphates are released, while sodium and chloride ions partially penetrate the cells (see Fenn, 1936, 1937b, 1938a, 1939). During "rest" an opposite motion of these ions is seen, as if against the concentration gradient, if it is assumed that the electrolytes are simply dissolved in the protoplasm. In addition, it can be shown by means of "tagged" atoms, that potassium, sodium and chloride ions may both penetrate the cells sufficiently rapidly, and leave them while the concentration differences inside the cell and outside it are maintained.

All these facts could not be explained by the membrane theory in its original form. It was necessary to assume the existence of a certain pumping mechanism which, at the expense of energy, pumped potassium into the cell and forced sodium and chloride ions outside. These assumptions were made by certain investigators and of necessity made the proposed structure of the membrane even more complicated. Thus, Lundegardh (1940) put forward the hypothesis that the cell surface was lined by long molecules or micelles in the form of a palisade (Figure 57) consisting of one or two layers. A part of these molecules had positive charges and the other, negative charges, these being arranged on the surface in a mosaic fashion. The mechanism of permeation of ions into the cell was thought to be as follows. First the ion combined with the end of the micelle which was part of the membrane, and the micelle then rotated through 180°; in this manner the attached ion was then at the other side of the membrane.
109 This mechanism may thus either pump in or remove from the cell these or other ions, with an expenditure of energy.

Krogh (1943) considered Lundergardh's theory highly speculative. Nevertheless he accepted it not only as the only satisfactory one, in his opinion, but even complicated it by assuming that the free ends of the rotating micelles possessed an affinity either for potassium or for sodium. Making a 180° turn the membrane molecules maintained the necessary ratio between the concentrations of these ions inside and outside the cell. But the nature of the forces rotating the membrane molecules, and the nature of the regulating mechanisms securing the constant ratios between the ionic concentrations of the protoplasm, remained unknown.

Another hypothesis was formulated by Rosenberg and Wilbrandt (1952). This dealt with the penetration of glucose into cells. According to the classical considerations of the membrane theory, sugars do not penetrate cell membranes since in a hypertonic solution they cause a stable decrease in the volume of the cells. Yet, glucose is one of the main sources of nutrition for the cells.

How can these two mutually exclusive facts be reconciled?

The authors of this theory assumed that glucose was transported into cells by means of enzymes. In their opinion, two enzymes located at opposite sides of the cell membrane acted conjointly. The sugar was temporarily transformed into a soluble form in the substance of the membrane and thus acquired the ability to diffuse through the membranes. This "transportable" form of sugar existed only within the membrane. The authors of the theory assumed that the permeating substance became "transportable" as a result of combination with another substance (or substances) which may be called "membrane carriers".

FIGURE 57. Structure of the protoplasmatic membrane (according to Lundergardh, 1940)

1-micelles; 2-their radicals; R^+ and R^--the positively and negatively charged radicals, respectively; OH^- and H^+-compensatory ions; A^- and M^+-exchanging ions.

The theory of Rosenberg and Wilbrandt seems to us as arbitrary and poorly based as that of Lundergardh. It is known that enzymatic processes are specific. Consequently, for each substance penetrating the cell there would have to be at least three specific substances in the membrane-two enzymes and one "carrier". Therefore the membranes would have to be saturated with these additional substances. This is not very probable, assuming the thin structure of the membrane.

These are the complicated, arbitrary and hypothetic considerations resulting from the membrane theory of permeability. Comparatively simple when it originated, it became more and more complex as a result of continuous new data which could not be reconciled with the original theory. For many physiologists who are not especially absorbed in the problems of cellular physiology, the term "semipermeable cell membrane" acquires a somewhat relative symbolic meaning, which they prefer not to invest with any concrete significance (Kan, 1939).

The Osmotic Properties of the Cell

One of the strongest arguments in favor of the membrane theory of permeability has always been that in strong solutions of substances which apparently do not permeate into the cell, the cells behave as tiny osmometers On measuring the osmotic pressure of the environment (P), the volume of
110 the cells (V) should change in inverse proportion to this pressure (PV = const).

In this respect the material first used in the study of permeability will be considered, as in plant cells with a large central vacuole. As shown by Pfeffer (1877) and by de Vries (1884), the volume of these cells in solutions of different osmotic pressures really obeys the law PV = const. However, on the basis of these old experiments it cannot be stated where in the cells "the elementary osmometers", i.e. the membrane, is located. Pfeffer and de Vries assumed that the membranes are located outside the cells at the border between the protoplasm and the environment. In this case the term cell permeability could really be used. However, results superficially similar to these could have been obtained if the semipermeable membranes were located at the surface of the vacuole within a special membrane, separable from the protoplasm and called "tonoplast". If this were so, then all the osmotic experiments would give data applicable only to the permeability of the tonoplast, which would regulate the permeation of substances into the vacuole but not into the cytoplasm.

An answer to this problem may be found in the very thorough studies of Höfler (1918, 1930-1934, 1939; Huber and Höfler, 1930). These authors

elaborated a method of differential determination of the volumes of the protoplasm and vacuole of plant cells. With this they showed that, following changes in osmotic pressure of the environment, only the volume of the vacuole obeyed the law of osmosis. In addition, Höfler found that many of the substances which did not penetrate through the tonoplast into the vacuole easily penetrated into the protoplasm. Consequently the conclusions on permeability based on so-called osmotic experiments can in no way be related to permeability of the protoplasm of plant cells.

Supplementary to these data, Höfler, together with the noted inventor of the micromanipulator Chambers (Chambers and Höfler, 1931), showed that the plant vacuole surrounded by the tonoplast may be easily isolated from the surrounding protoplasm, and that such an isolated vacuole changes its volume in different solutions, obeying the law of osmosis. All this supports the contention that the results of the classical experiments on plasmolysis of plant cells may be related mainly to the properties of the tonoplast and the vacuole, but not to those of the protoplasm.

The interesting data given by Maksimov (1946) also supports this contention. From the data in the table compiled by him (Table 24) it will be seen that the osmotic pressure of the protoplasmic sap is 2-5 times greater than that of the vacuolar sap. It is difficult to understand why water does not flow from the vacuole into the protoplasm. Obviously in the protoplasm of plant cells the amount of water is not regulated by osmotic forces. Maksimov and other authors (Maksimov and Mozhaeva, 1944a, 1944b; Mozhaeva, 1947, 1950a, 1950b; Shcherbakov and Semiotrocheva, 1953 and others) reached similar conclusions.

In this connection it was of interest to elucidate whether plant cells devoid of vacuoles obeyed the laws of osmosis.

Such a study was performed by Walter (1923) using vacuoleless cells of sporogenic threads of the alga Lemanea. The results showed that the changes in volume of these cells in sugar solutions did not correspond with
111 those which would be expected if the cells were surrounded by semipermeable membranes. Comparison of this author's results with data on changes in volume of nonliving models led Walter to the conclusion that the loss of water by cell protoplasm was a result not of osmotic processes, but of dehydration of lyophilic colloids, which are components of protoplasm. The principal difference between these two phenomena is that osmosis is possible only under conditions of impermeability of the substance in relation to the cell, while dehydration is possible under conditions of permeation. Consequently, a decrease, for example, of the volume of the vacuole in a hypertonic solution would be a proof that the substance did not enter into the vacuole. A decrease of the protoplasm volume would show that the substance did penetrate the protoplasm.

Together these facts force us to adopt a very careful attitude to all the data on permeability obtained by the plasmolytic method of vacuolated plant cells.

Rubinshtein, who is the leading advocate of the membrane theory, was forced to begin his address at the discussion on permeability in Moscow in 1935 with the following statement: "We are forced to abandon completely all the results obtained in studies of plant cells by the use of the osmotic method. As is well known, these were historically the basis of the contemporary theory of the semipermeable plasmatic membrane" (Rubinshtein, 1939, p. 9).

The position is much the same regarding the "osmotic" properties of animal cells.

Overton (1902) drew attention to the fact that animal cells were capable of losing water or swelling in solutions of different substances.

At the same time, under the influence of the studies of Pfeffer and de Vries, the opinion was firmly established that plant cells may be considered as osmometers, with the semipermeable membrane located at the external surface of the cell. We have already mentioned that this point of view turned out to be incorrect and that only the vacuole in plant cells works as an osmometer. This vacuole does not exist in animal cells. However, in Overton's times this was not known, and it was therefore natural for him to transfer the idea of "osmotic" properties of plant cells to animal ones. That is why the changes in volume of animal cells observed by Overton in various solutions were considered by him as osmotic phenomena caused by the presence of semipermeable membranes at the protoplasmic surface.

Table 24

Osmotic pressure (in atmospheres) in cells of leaves of different plants (according to Maksimov, 1946)

Sap	Beech	Wild vine	Cotton-plant
Protoplasmic	18-24	25	17-18
Vacuolar	5-8	5-7	9-10

112 As a result of this error by Overton, later researches were not valid. The error was augmented by the great authority of the botanists Pfeffer and de Vries, and by the impressive thoroughness of Overton, in whose works the permeability of more than 500 highly varied substances was studied. In addition, a concept was put forward which nicely unified the phenomena occurring in plants as well as in animals. Moreover, such important points in cellular metabolism as the entrance and exit of substances into the surrounding medium were reduced to a relatively simple physical phenomenon-diffusion through the membrane.

The main subjects of Overton's studies were the skeletal muscles of the frog, and the main method was to weigh the muscles before and after they were kept in one or another solution. On the basis of this data, Overton drew conclusions relating to the permeability of various substances into the muscle fibers, and he checked the applicability of the law of osmosis to animal cells.

The loss of water by muscles in hypertonic solutions, and their swelling in hypotonic solutions, were less than expected from the equation PV = const. Overton made an arbitrary assumption that 35% of the water in the cell was bound by protoplasmic colloids and therefore did not participate in osmotic phenomena. Assuming this, the obtained experimental data were seen to be close to the theoretical ones.

Later, Hill (1930) attempted to determine the amount of bound water in the protoplasm of muscle fibers by the use of a thermoelectric battery

which he constructed, obtaining a quite unexpected result. According to his data, 95% of the water in muscle fibers was in free form and only 5% bound*. Under such conditions, osmotic experiments with muscles should not have resulted in deviations from the law of osmosis. However Hill himself, (1930), using the same tissue—frog muscles—obtained a 100% deviation from the osmotic law. This deviation the author explained not by the presence of bound water (according to his data there was no such bound water in muscles), but by the fact that the muscles were supposedly damaged during preparation and that 25% of the fibers had lost their osmotic properties. This assumption was not confirmed and was as arbitrary as that of Overton with his concept of 35% of bound water.

Later authors, who proved the applicability of osmotic laws to animal cells, held much the same views. They studied the change in cell volume in hypo- and hypertonic media, these changes usually being smaller than set out theoretically. A certain percentage of bound water was assumed and thus the gap between the data obtained and the theoretical requirements was filled. It would seem that the same amount of bound water would be obtained no matter what solution of impermeable substance was used. In his book, Ponder (1934, p. 119) gave data on the increase of volume of rabbit erythrocytes when submerged in different hypotonic media. The author calculated from these data the amount of bound water which seemed to vary, depending on which substance was used for its determination. If the experiment was performed with a solution of NaCl, the results were 30–50%; however, when glucose was used, the figure was 20–36%. It is extremely difficult to understand these results from the point of view of the osmotic theory.

113 On the basis of osmotic experiments, Ege (1921a) proved the complete impermeability of rabbit erythrocytes to glucose, sodium chloride and sodium sulfate. However, in isotonic solutions of these substances the value of the erythrocyte volumes differed. The results of Ege's experiments clearly contradict the osmotic theory.

According to the data of Schiødt (1931), obtained with erythrocytes of dogs, and Ørskov (1946) with human erythrocytes, the correction (b) which has to be introduced into the osmotic formula P (V-b) = const., and which supposedly corresponds to the amount of bound water in the protoplasm (plus the dry residue), itself changes in relation to the osmotic pressure of the medium. Thus, at low concentrations of NaCl, almost all the water of the cell is osmotically active; at higher concentrations, however, half of the water is in the bound form. These phenomena are also difficult to explain from the point of view of the osmotic theory.

Many "osmotic" experiments were performed with ova cells of marine animals (McCutcheon and Lucké, 1927, 1932; Lucké and McCutcheon, 1927, Page, 1927; Ephrussi et Neukomm, 1927; Bialaszewicz, 1929, 1933; Lucké, 1931, 1940; Stewart, 1931a, 1931b; Stewart and Jacobs, 1932, 1936; Dorfman, 1933; Lucké, Larrabee and Hartline, 1935/36; Lucké, Ricca and Hartline, 1936; Lucké, Hartline and Ricca, 1939, and others).

However, there are no data showing that the water balance of the ovum cells is regulated by osmotic factors. In almost all the experiments cited, and in the above-mentioned studies with muscles and erythrocytes, the authors used the formula (V-b)P = const, being a correction corresponding, in their opinion, to the osmotically inactive, bound water. Since the value

---

* Troshin in his book (1956) points out the source of Hill's error.

value of this correction was arbitrarily chosen by the author himself, the agreement between the theory and the experiment is no more convincing.

Finally, the interesting work of Vasil'ev (1922) should be mentioned. He determined the changes in the nerve diameter in isotonic solutions of mineral salts, acids and alkalis, using an ocular micrometer. The results showed that the volume of the nerve changed to different extents in these solutions. In acids and in alkalis it increased, while in salt solutions it decreased. The water-reducing effect of salts varies within the series $KCl<NaCl<CaCl_2$. It is quite obvious that these phenomena cannot be explained by osmotic phenomena but they are well explained by the assumption of the colloidal-chemical phenomenon of swelling and shrinking. The author himself reached the same conclusion.

As seen from this short review of literature concerning the water balance of different animal cells, it is doubtful whether the water equilibrium between the protoplasm of such cells and the surrounding environment is determined by osmotic forces based on the cell envelope of semipermeable membrane. This fact emphasised the necessity to check again the applicability of the law of osmosis to animal cells (Nasonov and Aizenberg, 1937).

At first we chose calf muscles of the frog for study. The change in the water content was determined not by the decrease or increase in weight, as was done by Overton (1902), but directly by changes in volume. The volume was determined by the column of liquid displaced after immersion of a great number of muscles in long, narrow chemical burettes. By doing this we removed a source of error connected with the drying of the muscles before weighing, and with corrections for the specific gravity of the dissolved substances. The muscles were placed in solutions of the substances
114 studied at different concentrations (prepared in Ringer's solution) and their volumes measured at given time intervals until final equilibrium occurred, i.e. after 2 to 3 hours. After the experiment, the excitability of the muscles was always checked by electric stimulation.

In the choice of substances we were guided mainly by the classical work of Overton. Thus, almost all the substances studied by us were previously studied by him. Overton established the famous series of permeability, at the end of which sugars and polyhydroxy alcohols were listed as substances which absolutely do not penetrate the cell. Overton was quite correct in insisting on complete impermeability of sugars, from the point of view of osmotic considerations, since in his experiments the muscles were not deplasmolyzed in sugar solutions within six days. In order to obtain an osmotic effect of equal strength, many other substances had to be studied at higher concentrations, which according to Overton, explained their partial permeability, although these substances also did not cause deplasmolysis. The effect of the following substances was studied: among the macromolecular colloid substances—egg albumin, gum arabic, and peptone; among the disaccharides—saccharose and lactose; among the monosaccharides—glucose and galactose; among aminoacids—alanine, followed by urea and glycerol. According to Overton, only the two latter substances among those studied penetrated the muscle fibers.

Using the above method of measuring volumes, we were able to construct a graph of changes in volume of muscles with time for each concentration of the substances studied. As seen from Figures 58 and 59, during the first hour a comparatively rapid loss of water occurred, followed

by a slow attainment of the final equilibrium by the muscle. For the comparison of the relative effect of substances on the volumes, we chose the time of 2 hours and 30 minutes.

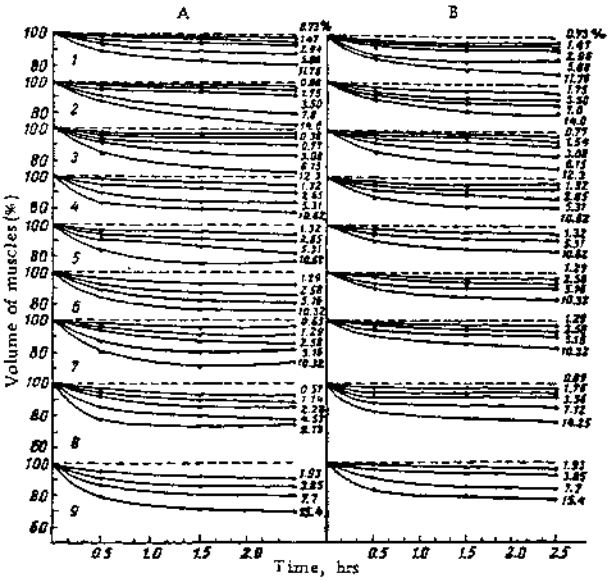


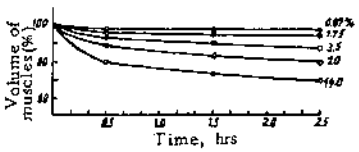
FIGURE 58. Change in the relative volumes of (A) living and (B) killed muscles in the presence of nonelectrolytes (according to Nasonov and Aizenberg, 1937)

1—gum arabic; 2—egg albumin; 3—dextrin; 4—saccharose; 5—lactose; 6—glucose; 7—galactose; 8—alanine; 9—glycerol.

115 Figure 60 shows a series of curves corresponding to each of the substances studied. The abscissa shows the logarithms of the molar concentrations of these substances, in order to facilitate comparison, while the ordinate gives the volume of the muscles, measured after 2 hours and 30 minutes, in one or another solution. In all cases the initial volume measured before immersion in the test solution was accepted as 100%. In the following series the curves are distributed according to the strength of water removal from the muscles: urea<glycerol<alanine<glucose, galactose<saccharose, lactose<dextrin<gum arabic<egg albumin. In a group of curves from sugars to urea, the series corresponded to Overton's permeability series, according to which sugars and aminoacids did not penetrate at all into living muscle fibers. The remaining substances influenced the muscle volume in a degree inversely proportional to the ease with which they permeated the membrane.

It is well known that living muscles contain 80% water. An 8% solution of saccharose (0.25 M for amphibian muscles) added to an isotonic solution

of Ringer (0.23M), increased the osmotic pressure of the medium approximately twofold. If the semipermeable membranes of the muscle fibers were really impermeable to sugars and salts, the muscle fibers would lose 40% of their volume. In reality, however, muscles, while alive, lost only 16.7%, i.e., 2.4 times less than required by the membrane theory. We have already mentioned that Overton, in order to overcome this difficulty, assumed that 35% of the water in the muscle fibers was bound and therefore osmotically inactive. We have also seen that other investigators, too, who worked with erythrocytes, ovum cells etc., explained the discrepancies between the experimental facts and the requirements of the theory in this way. However, there is a possibility for the objective solution of the problem of whether water is really bound.



117

FIGURE 59. Change in relative volume of living muscle in peptone solutions (according to Nasonov and Aizenberg, 1937)

If sugar does not penetrate the muscles, and if the removal of water is due to osmosis, it is understandable that there can be no other substance which at concentrations isotonic with sugar would remove more water than it does. However, it follows from Figure 60 that the curves of water removal for dextrin, albumin and gum arabic are located considerably to the left of that of sugar. This indicates that the muscles lost approximately similar amounts of water in 0.25 M solution of saccharose, 0.04 M solution of dextrin and 0.002 M solution of egg albumin and gum arabic. In other words, in order to obtain the same reduction in volume of living muscles, it was necessary to use a dextrin solution six times weaker than the sugar solution, while egg albumin or gum arabic caused the same reduction of water at concentrations 125 weaker than that of sugar.

It is clear that such an excessive water removal cannot be caused by osmosis. Indeed, an isotonic concentration for muscles corresponds approximately to that of a 0.23 M solution. An 0.003 M solution of egg albumin in an isotonic Ringer's solution has an osmotic pressure corresponding to 0.23 + 0.003, i.e. 0.233 M solution. Consequently, even if it is assumed that the osmotically active water in muscle comprises 100% of its volume, the volume of the muscle in such a solution should decrease 0.233/0.230 (= 1.013 times), i.e., by 1.3%. Actually, however, it decreased by 21%, as can be seen from Figure 60.

Thus, if in solutions of saccharose, muscles lose less water than required by the membrane theory, in solutions of albumin, gum arabic and dextrin, they lose more than theoretically expected. Obviously it is not possible to explain both deviations from the theoretical requirements by assuming the presence of bound water.

It is interesting that the facts (of considerable reduction in volume of muscles in dextrin and albumin solutions) were known to Overton, although he mentioned albumin only indirectly, giving no numerical values. "These apparent contradictions caused me many headaches for a long time"*.

---

* "Diese scheinbare Widersprüche die mir zeitlang viel Kopfzerbrechen verursachten" (Overton, 1902, S. 237).

In order to save the membrane theory, Overton proposed the following explanation: in his opinion colloid solutions do not penetrate through cell membranes, nor even through the connective tissue membrane (perimysium) enveloping all the muscle, while salts diffuse freely through this membrane. On the basis of this assumption it may be deduced that although the external colloid solution may be weak, it can extract all the liquid from the intercellular space of the muscle.

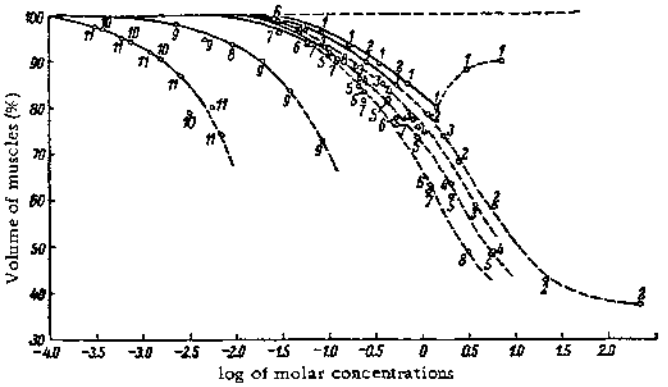


FIGURE 60. Effect of nonelectrolytes on the volume of living muscles (according to Nasonov and Aizenberg, 1937)

1—urea; 2—glycerol; 3—alanine; 4—glucose; 5—galactose; 6—saccharose; 7—lactose; 8—peptone; 9—dextrin; 10—egg albumin; 11—gum arabic. The concentration regions in which the muscles still maintain their excitability are designated by solid lines.

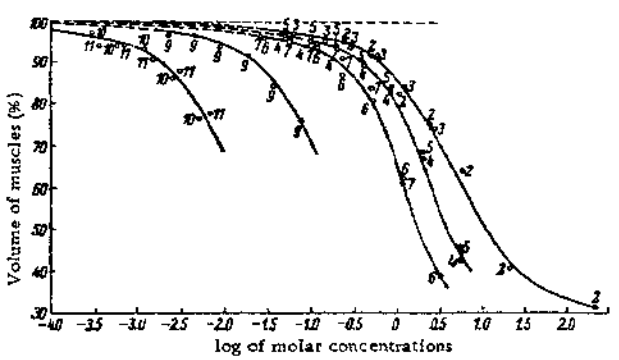


FIGURE 61. Effect of nonelectrolytes on the volume of killed muscles (according to Nasonov and Aizenberg, 1937)

Legend as in Figure 60.

In order to check the correctness of Overton's explanation, analogous experiments were performed with ova which develop in sea-water, where osmotic "suction" of water from the intercellular spaces does not apply (Aizenberg, 1939). Nonfertilized egg cells of the starfish (Asterias rubens) and the sea worm (Pectinaria hyperborea) were used in these studies. Their changes in volume were studied by measuring their diameters under the microscope. The water-removing effect of solutions of glycerol, monosaccharides, disaccharides and dextrin dissolved in sea-water was studied. The results of experiments performed according to the same scheme as those with muscles, are illustrated in Figures 62-64.

Figure 62 illustrates the gradual approach of the volume of egg cells to the equilibrium state after immersion in nonelectrolyte solutions of various concentrations. Here, too, all the solutions caused a removal of water, the equilibrium being attained sooner than in muscles (after 0.5-1 hour). As in muscles, deplasmolysis was not observed while the egg cells
118 were submerged. From this it would be concluded, according to the membrane theory that these ova were completely impermeable to all the substances studied. Under such conditions all these substances in isomolar concentrations should have removed water to the same extent.

However, as seen from Figures 63 and 64, the substances used form a series in relation to their strength of water removal: dextrin>lactose, saccharose>glucose, galactose>glycerol. Here, as in the case of muscles the substances in the righthand part of the series (for example dextrin) remove water in greater amounts than required by the theory. The excessive removal of water in this case cannot be explained by osmotic forces, since there are no intercellular spaces here, about which Overton wrote while attempting to explain the excessive removal of water from muscles by solutions of macromolecular compounds. At the same time, there is no doubt that the cells used were fully alive. This was proved by the fact that after being rinsed in pure sea-water, the egg cells used in the experiments were fertilized and were shown to be capable of normal fragmentation.

Thus, isomolar solutions of the investigated surface-active nonelectrolytes formed the same series in relation to their water-removing force in respect to muscles and egg cells as the series in respect to their molecular weight: gum arabic (37,037), egg albumin (34,000)>dextrin
119 (2,736)>saccharose (342), lactose (342)>galactose (198), glucose (198)>glycerol (92), alanine (89)>urea (60). It has been shown that these substances also give the same series in respect to the strength of water removal from muscles killed by 96° ethyl alcohol or by 0.2% NaOH. The results of the experiments with killed muscles, performed similarly to those with living ones, are illustrated in Figure 61. Killed muscles, submerged in nonelectrolyte solutions, lost approximately the same percentage of water, more or less at the same rate as living muscles, and they showed no deplasmolysis.

Comparison of Figures 60 and 61 shows the extraordinary resemblance between the dehydration curves of the nonelectrolyte series for live and killed muscles. However, the latter comprise a well-known membraneless colloid system, and therefore cannot be considered as tiny osmometers. It is quite obvious that this is a colloid-chemical dehydration and not an osmotic removal of water. This is confirmed by the fact that in respect to the dehydrating capacity of many nonsurface-active nonelectrolytes, the same series was obtained for such a simple and well-known colloid system as swollen gelatine, by Weber and Lederer (1936) and by Nasonov, 1938.

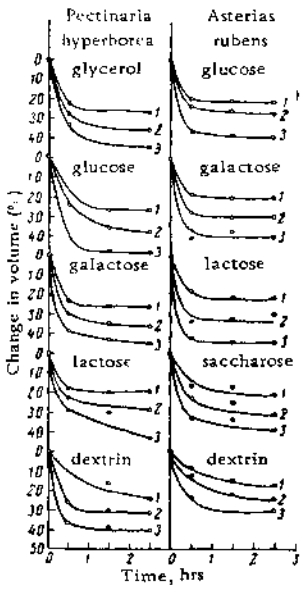


FIGURE 62. Change in volume of ova of sea animals, in relation to the concentration of the nonelectrolytes in the medium (according to Aizenberg, 1939)

Concentration of nonelectrolytes: (1) 2.5%; (2) 5%; (3) 10%.

All the above data lead to only one conclusion: removal of water from animal cells by solutions of different nonelectrolytes is not an osmotic process but a colloid-chemical dehydration. From this follows a very important conclusion for the theory of cell permeability: decrease in volume of animal cells on immersion in solutions of these or other substances shows that these substances penetrated the cells, because colloid dehydration may occur only under the condition of soaking the colloid system by the dehydrating solution. Thus, from the point of view of the osmotic theory, the same phenomenon—decrease in volume—indicates that the substance did not penetrate, while from the point of view of the colloid theory the opposite is true.

If our data are correct, as is easily checked, it then follows that all the substances studied penetrate comparatively rapidly into the animal cells independently of the size of their molecules, starting from urea (molecular weight of 60) up to such substances as egg albumin, (molecular weight of 34,000*), the entrance of the substances being accompanied by exit of water from the protoplasm. This was proved by Troshin (1953) by direct analyses of a number of substances. Figure 65 illustrates permeation of galactose into erythrocytes (b) with simultaneous loss of their water (a). Both processes are parallel to one another and therefore the process is not osmotic. There is also no deplasmolysis. The same is seen in Figures 66 and 67.

In connection with the above, the widely accepted concept "isotonic solution" used in contemporary physiology should be revised. This term denotes a solution the total molar concentration of which equals the sum of molar concentrations of all substances dissolved in the cell. This term has a meaning only under the condition that the substances contained in the solution surrounding the cell do not pass through the semipermeable cell membrane, therefore creating an osmotic pressure which tends to remove water from the protoplasm. It is also assumed that the substances in the cells are in a free dissolved state and also do not pass through the cell membrane to the outside, with a resultant osmotic pressure inside the cell, tending to draw water from the surrounding fluid**. The equality of the osmotic pressures inside and outside the cell supposedly ensures the water equilibrium.

---

* Many other proofs are now available for the penetration of protein molecules into animal cells (Meshcherskaya, 1931; Oparin and Yurkevich, 1949; Yurkevich, 1954, and others).

** It will be shown later that this assumption is erroneous.

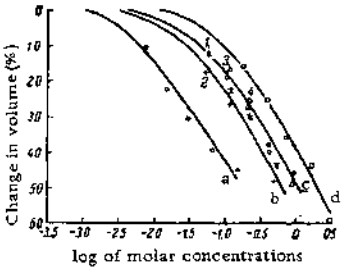


FIGURE 63. Change in volumes of ova of Pectinaria hyperborea, in relation to the concentration of nonelectrolytes in the medium (according to Aizenberg, 1939)

a—dextrin; b—lactose (1) and (2)—saccharose; c—glucose (3) and (4) galactose; d—glycerol.

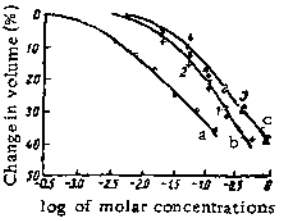


FIGURE 64. Change in volumes of ova of Asterias rubens in relation to the concentration of nonelectrolytes in the medium (according to Aizenberg, 1939)

Legend as in Figure 63.

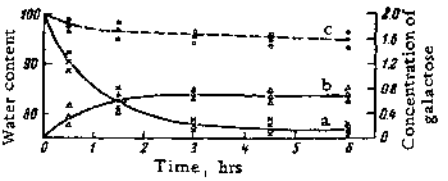


FIGURE 65. Change in water and galactose content in rabbit erythrocytes, placed in a 2% solution of galactose prepared in Ringer's solution (according to Troshin, 1953)

a—decrease in water in the erythrocytes (percent of control); b—uptake of galactose by erythrocytes (in percent per 100 g of cellular water); c—decrease in galactose in the medium (percent).

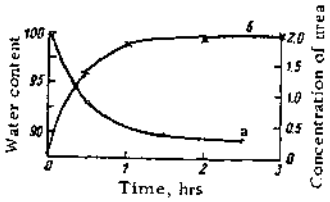


FIGURE 66. Change in volume of frog muscles in a 2% solution of urea

a—dehydration of muscles (percent of control); b—accumulation of urea in muscles (in percent per 100 g of tissue water; concentration of initial urea solution—4%). (a—according to Nasonov and Aizenberg, 1937; b—according to Troshin, 1953).

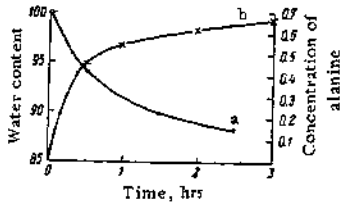


FIGURE 67. Change in volume of muscles in a 1.69% solution of alanine (according to Troshin, 1956)

a—dehydration of muscles (percent of control); b—accumulation of alanine in muscles (in percent per 100 g of tissue water; concentration of initial alanine solution—2%).

121 We recently reached the conclusion that this concept is incorrect; that all the substances do penetrate into the cells and that the water equilibrium is established not osmotically, but by chemical forces which cause colloid swelling. Consequently, the expression "a solution isotonic for cells" has no scientific value and may, in many cases, mislead the investigator.

Chapter 2. Basis of the Sorption Theory of Cell Permeability

Penetration of Nonelectrolytes into the Cells from the Point of View of Diffusion and Distribution Phenomena

In terms of the membrane theory of permeability, protoplasm may be considered as an aqueous solution of different substances surrounded by a semipermeable membrane. If the cell is surrounded by a solution of any substance not present in its protoplasm, or which is present there at a lower concentration, this substance will tend to diffuse into the cell due to the presence of a concentration gradient. A membrane lies in the path of this diffusion, which either does not let the substance through at all or slows down its diffusion to a greater or lesser degree. The permeability of substances through the membrane should be determined by the velocity of its diffusion. Finally, the concentration of the substance inside and outside the cell should become equal. Figure 68 A illustrates curves showing the increase in concentrations of the substances permeating into the cell, related to time. The final level should in all cases be the same (equal to the external concentration), but the rate of attainment of this level may differ. This may be checked by analyzing the cell content at different time intervals after placing the cells into various solutions.

Unfortunately, the original investigators made very little use of such a direct but quite complicated method of study of permeability and, following the example of Overton, they mainly used the simpler but indirect osmotic method. Later, the method of direct analysis became more generally used, the subjects being mainly cell suspensions, such as erythrocytes, yeasts, etc. It became gradually clear that a simple picture such as the one described in Figure 68 A was never actually observed, but the reaction taking place was rather that illustrated in Figure 68 B. The majority of the substances studied penetrated the cell at approximately the same rate, but the intracellular equilibrium concentration only rarely coincided with the external one (Figure 68 B, 3). For some substances this equilibrium concentration was lower than the external one (Figure 68 B, 1, 2), while for others it was higher (Figure 68 B, 4). Thus, the difference in the nature of penetration of a substance was determined not by the rate of penetration but by quite another index—the established level.

Again in terms of the membrane theory, an equilibrium level exceeding the external concentration might be explained by absorption of the penetrating susbstance onto surfaces of cell colloids. But a level lower than the external concentration was quite inexplicable in this way. It required special explanations, especially in cases where the penetrating substance was not decomposed and did not enter into any combinations within the cell. This lower level could be explained by the fact that a part
122 of the water in the cell was bound and did not therefore serve as a solvent. However, the percentage of the excess of the external concentration of the substance above the intracellular one should always have been the same, independently of the substance whose penetration is studied.

In view of the fundamental importance of these phenomena for the understanding of permeability, a number of examples will be discussed and analysed. First, the simplest, i.e. permeability of cells in respect to surface-inactive nonelectrolytes. Later, those experiments in which the direct method of chemical analysis was employed will be considered.

Many experiments were performed on the permeability of erythrocytes. Originally, the investigators mainly used the osmotic method, assuming that monosaccharides, disaccharides, and polysaccharides did not penetrate at all into erythrocytes (Grijns, 1896; Hedin, 1897, 1898; Masing, 1913, 1914a, 1914b; Kozawa, 1913, 1914; Ege, 1920a, 1921b; Mond and Hoffmann, 1928; Fleischmann, 1928; Mond, 1930, and others). However, the use of the direct chemical analysis often led to contradictory results. The proponents of the membrane theory objected to the chemical method which supposedly gave distorted results since substances adsorbed on the surface of cells were, according to this method, considered as having penetrated the protoplasm (Fleischmann, 1928, Mond and Hoffmann, 1928; Mond, 1930; Gelhorn, 1932; Rubinshtein, 1947, and others). However, against this assumption is the fact that the increase in permeability to fermentable sugar detected by the chemical method is always accompanied by a corresponding increase in glycolysis, and such a complicated process cannot be performed only on the cell surface (Kolotilova and Engelhardt, 1937; Brooks, 1947, and others). In addition, the process of adsorption on surfaces of suspended particles should be completed in a matter of seconds. As far as the erythrocytes are concerned, the equilibrium between the external concentration and the content of the substance

in the cells is established within several hours (Figure 69), completely excluding the phenomenon of adsorption to external surfaces of erythrocytes.
123 Thus, the direct chemical analysis is after all the best of all known methods used in the study of permeability of animal cells.

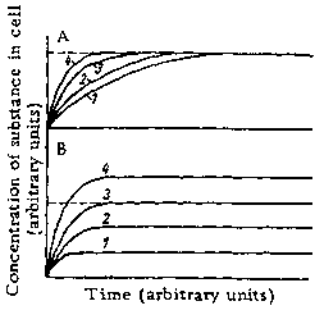


Figure 68. Schematic presentation of penetration of various substances (1-4) into the cell

A-with the membrane mechanism; B-with the sorption mechanism. The concentration level of the substance in the surrounding medium is indicated by the broken line.

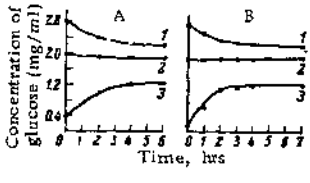


FIGURE 69. Uptake of glucose by rabbit erythrocytes in the presence of enzyme poisons (according to Kolotilova and Engelhardt, 1937)

A-fluoride sample; B-iodoacetate sample. 1-sugar in the liquid part (in the medium); 2-sugar in suspension; 3-sugar in erythrocytes.

We have already mentioned that on penetrating into erythrocytes, sugars reach a definite concentration level in the protoplasm which is always lower than that in the external solution. The ratio between the internal level established (C_c), to the external one (C_s), was designated as the distribution coefficient (Q) and was used by certain investigators as a measure of permeability, although it is quite obvious that the distribution coefficient has nothing in common with permeability and diffusion rate through the membrane.

Examples will be given of the values of $\frac{C_c}{C_s} = Q$ for various substances and objects. Thus, Masing (1914a) found in the case of penetration of dextrose into human erythrocytes that Q equals 0.52-0.70. According to Ege (1920a, 1921a, 1921b) the penetration of glucose into dog erythrocytes is characterized as Q = 0.33; in human erythrocytes, Q=0.75. Folin and Berglund (1922a) and Wu (1922) found this coefficient for human erythrocytes to be close to unity (Q = 1.0). Swedberg (1933), using the method of direct chemical analysis, determined the sugar content in plasma and erythrocytes of different animals. He gave the following ratios of these two values: for the dog—0.21, guinea pig—0.23, ox—0.26, rabbit—0.15, monkey—0.54.

Similar results were obtained by Woodhaus and Pickworth (1932), who showed that the final concentrations of glucose and urea in erythrocytes were considerably lower than those in the surrounding medium. The distribution coefficient for urea was approximately 0.60 and for glucose-0.40. According to these authors, such distribution of substances depended to a considerable degree on the selectivity of the protein substrate of the erythrocytes.

There are many similar studies in the literature. In all of them the coefficient of distribution of sugars between the protoplasm and the environment is always given as less than unity. We have already said that in relation to fermentable sugars, this may be explained by the fact that the decrease

sugar within the cells (as a result of splitting) is not sufficiently rapidly replenished by diffusion from the external medium. However, for nonfermentable sugars, too, the value of Q is always less than unity. In addition, in the works of Engelhardt and Kolotilova (1936), Kolotilova and Engelhardt (1937) and Kolotilova (1937), it was shown that on treating erythrocytes with fluoride or monoiodacetate (which stop glycolysis), penetration of sugar into the cell proceeded quite rapidly.

Figure 69 shows that after 2-4 hours the entrance of glucose into the cells ceased, and the sugar concentration established in the cell was half that of the medium. According to the authors' data such nonfermentable sugars as galactose, arabinose, and xylose also penetrated the erythrocytes and in these cases the external concentration was always higher than the intracellular one. Finally, there are papers in which penetration of sugars into erythrocytes has been convincingly established. Keth and Power (1937) introduced saccharose into human blood and they observed that after 6 hours the concentration of the sugar in the plasma was 3 times as high than that in the cells. Similar results were obtained by Vorob'ev
124 (1939), who studied the permeability of stored human erythrocytes in respect to saccharose.

It is well known that the main source of energy for the work of muscles is the splitting of carbohydrates; therefore sugar should freely penetrate the working muscle. However, Overton (1902), on the basis of his osmotic experiments, reached the conclusion that muscles are completely impermeable to sugars. Overton's point of view was shared by Hill (1930, 1935) and by Höber (1945). Strictly speaking, all the adherents of the membrane theory should share this point of view. Overton drew his conclusions from the fact that in hypertonic sugar solutions muscles lose water and do not return to their initial volume before death. In spite of this, direct chemical analysis indicates penetration of sugars into skeletal muscle fibers and into the heart muscle. In these cases, too, an equilibrium concentration lower than that of the surrounding medium is established. Thus, Schulze (1927) observed that the intramuscular concentration of glucose reached 36% of the external one, while Eggleton's (1935) figures were 16-27%. It is true that both authors assumed that the sugar penetrated only into the intercellular spaces; however, this assumption was certainly incorrect, since according to recent data, these spaces in muscles do not exceed 9% of the total volume of muscles. The permeability of muscles to mono-, di-, and polysaccharides was also demonstrated in the studies of Krogh and Lindberg (1944), Gzhatskii and Vandokanti (1947); Hetenyi, Issekutz et al. (1953). In all these experiments, direct chemical analysis of the medium and of the muscle cells was the method used.

Kamnev (1938), in a very thorough review of the permeability of skeletal muscles, studied the penetration of nonfermentable sugars—galactose and saccharose. He placed frog muscles in a relatively small amount of sugar solution in Ringer's liquid, and assessed penetration into the muscles by the decreased concentration in the surrounding solution. The penetration of saccharose from a 1% and a 4% solution, and of galactose from a 2% solution, were studied. The results of the experiments are described in Figure 70A, which shows the increase in concentration of the sugars within the cell with (a) its decrease in the surrounding solution, and (b) time. It can be seen that both sugars penetrated equally rapidly into

the cell and reached a certain concentration level in the protoplasm after 2-3 hours (b). This concentration never approached the level of the external solution (a), and comprised only a certain fraction of it. For galactose this was 42.1% of the external concentration, for 1% saccharose—32.7%, and for 4% saccharose—28.4%. There is no doubt that the sugars penetrated into the protoplasm of the fibers since the intercellular spaces in muscles comprise only 9% of the total volume of muscle. The low concentration level also cannot be explained by decomposition of the sugar molecules in the fibers because the sugars used were nonfermentable. This low level cannot be explained by the presence of bound water because in that case the percent of decrease should always have been the same. The author himself concluded that the amount of sugars in the fiber was determined not by a membrane mechanism but by the solubility of these substances in the protoplasm of muscles. The latter the author considered as possessing a dissolving capacity different from that of the water in the surrounding solution.

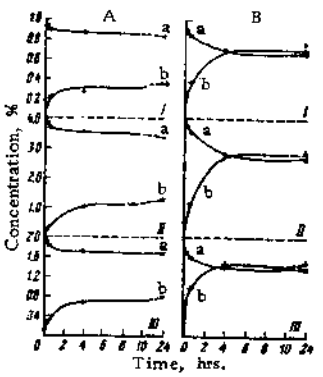


FIGURE 70. Entrance of sugars from the surrounding medium into (A) living and (B) killed skeletal muscles of the frog (according to data of Kamnev, 1938; from Troshin, 1956)

a—decrease of sugar in the medium; b—uptake of sugar by muscles. Initial concentrations of sugars in solution: I—1% solution of saccharose; II—4% solution of saccharose; III—2% solution of galactose.

Figure 70B shows that penetration of these sugar solutions into killed muscles presents a fundamentally different picture. The sugars penetrated the killed muscles at approximately the same rate as into living ones*; however, the final concentration levels of the sugars inside and outside the cell became equal in all cases (the slight excess of concentration inside the dead muscles may be explained by a slight adsorption of the sugars onto micelles of the dead protoplasm).

While in the case of surface-inactive substances, as for example sugars or monohydroxy alcohols, the equilibrium in the cells is established at a higher concentration of these substances in the medium ($Q = C_{protoplasm}$ $C_{medium} < 1$); in the case of surface-active substances which Overton considered to be readily permeable, an inverse relationship exists. The concentration inside the cell may be higher than that in the medium ($Q > 1$). Thus, Lazarev and Nusel'man (1932) and Brusilovskaya (1939, 1947) showed that ether, chloroform, and other narcotics, on entering the cell (erythrocytes) may reach concentrations considerably higher than the external ones. Fabre and Fredet (1925) found that on injection of veronal into the blood a ratio of 1.88 of veronal concentrations in erythrocytes and in serum was established.

---

* If permeability denotes the rate of penetration of a substance into the cells, then, on the basis of the above said one would draw the untenable conclusion that permeability of living and dead muscle is very similar.

Obviously, on entering the cell, the surface-active substances are adsorbed onto micellar surfaces, due to which a high concentration of these substances is established in the protoplasm.

Analysis of Distribution Curve. Studies by Troshin with Nonelectrolytes

A thorough study of the effect of various substances, and especially of nonelectrolytes, on the water content of animal cells, led us to the conclusion that this phenomenon is not based on osmosis but on colloidal-chemical phenomena of swelling. This in its turn led the so-called osmotic method of study of permeability to be abandoned on the grounds that it was based on false assumptions and gave erroneous results.

126 In this respect, the most reliable method is the direct chemical analysis of the cells and their environment. However, the few studies of this kind, as can be seen from the above review, suffer from serious shortcomings. The authors never gave any proof that they dealt with established equilibria. Another even more serious shortcoming of these studies is the fact that they used isolated, sometimes arbitrarily chosen, concentrations of substances, while in order to assess the nature of the permeability process it is necessary to study a series of concentrations. This permits the construction of a distribution curve for the substance between the cell and its environment

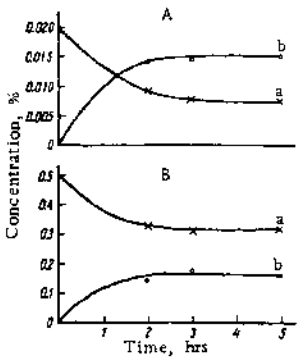
Studies of this kind were performed by Troshin, in all cases by a similar method. At first he determined the intercellular concentration of the investigated substance in relation to the environment, after equilibrium was established following a time interval. This was done with a series of increasing concentrations, starting with very weak ones, and ending with the strongest solutions not yet harmful to protoplasm. Subsequently, on the basis of the data obtained, a curve was drawn showing the relationship of the intercellular concentration of the substance (C_c calculated for the intracellular water) to its concentration in the surrounding medium (C_s).

Figure 71 illustrates the penetration of a nonfermentable sugar—galactose—into rabbit erythrocytes. Equilibrium concentration was reached in the erythrocytes in 2-3 hours. Obviously this was not due to adsorption onto the cell surface because this would have been established within a few seconds. However, the ratio between this equilibrium concentration (C_c) and the surrounding one (C_s) clearly depended on the concentration of the surrounding solution. In a weak solution (Figure 71, A), the concentration in the protoplasm was higher than in the environment, while in a strong solution (Figure 71, B), the reverse was true.

This is especially well seen from the data in Table 25, where with increase in concentration of galactose the coefficient $Q = \frac{C_c}{C_s}$ decreased to less than 1. Figure 72 was constructed according to the data in Table 25. The curve ob describes the relationship of the sugar content of the erythrocytes to its concentration in the external medium.

If a simple aqueous solution were present within the cells, the concentration of sugar in the erythrocytes would correspond to the straight line oa

(at an angle of 45° to the abscissa). However, the curve ob is far from
127 representing a simple dependence, as postulated by the membrane hypothesis. At first the curve rises steeply, being located above the bisectrix oa. This means that the cells take up the sugar from weak solutions at a higher concentration of the medium. Further, the curve ob intersects with the bisectrix and runs parallel to it. This means that the sugar diffusing from stronger solutions enters the cell at a lower concentration than that in the surrounding medium. The curve itself straightens out in this region, and continues at an angle to the abscissa smaller than 45°. If in the initial part the curve ob is similar to an adsorption curve, in the more distal regions it is a straight line similar to the distribution curve between two solvents, according to the law of Henry.



128 FIGURE 71. Disappearance of galactose in the medium, and its uptake by rabbit erythrocytes. (according to Troshin, 1956)

A—weak initial concentration in the medium; B—strong initial concentration in the medium. a—disappearance of the sugar from the medium; b—uptake of sugar by erythrocytes.

Troshin analyzed the curve and showed that it indicates two processes taking place in the cells: first, solution of the permeating substance in the dispersion medium of the protoplasm, and second, its adsorption onto surfaces of colloid particles. At the beginning adsorption prevails. Later however, when the surfaces of the micelles are saturated by the substance, adsorption gradually ends, and dissolution begins.

Insofar as the slope of the straight line corresponding to the process of solution is less than 45°, it may be concluded that the solubility of the substance in protoplasm is lower than its solubility in water*. The combination of these two factors—adsorption and low solubility—caused the distribution of the substances between the cell and the environment (Q), as observed in the experiment. It follows that the total amount of sugar in the protoplasm (C_c) consists of sugar dissolved in the protoplasm water (C) and of sugar adsorbed (A), or

$$C_c = C + A \qquad (1)$$

It may be assumed that the properties of the protoplasm water as a solvent (within the concentration range of the sugar employed) do not change in the surrounding liquid. K designates the distribution coefficient, in the equation of Henry, between the surrounding water and the water of the protoplasm. C designates the concentration of the sugar in the surrounding medium. The equation of Henry is then obtained

$$C = C_s K \qquad (2)$$

---

* It will be shown later that water of coacervate systems shows just such a low capacity for solution.

Substituting in equation (1) the value of C from equation (2), we obtain the equation:

$$C_c = C_s K + A \tag{3}$$

In this equation, A (adsorbed sugar) is not a constant value, but depends on the concentration of sugar dissolved in the protoplasm water (C), which according to equation (2), equals $C_s K$. The dependence of A on $C_s K$ may be expressed by the Langmuir equation for a nonpolar adsorption:

$$A = A_\infty \frac{C_s K}{C_s K + a} \tag{4}$$

where A_∞ is the limit of adsorption, and a is a constant characterizing the slope of the adsorption curve.

Substituting for A in formula (3) its value from equation (4) we get:

$$C_c = C_s K \left(1 + \frac{A_\infty}{C_s K + a}\right) \tag{5}$$

At a sufficiently high concentration of sugar in the equilibrium liquid, when a becomes so small in comparison with $C_s K$ that it can be neglected, equation (5) acquires the following form:

$$C_c = C_s K + A_\infty \tag{6}$$

This is the equation of the straight line db (Figure 72) inclined at a certain angle to the abscissa. This straight line intersects with the coordinate axis at the point d, the sector od equalling A_∞. In this way the adsorption limit for the substance under study can be graphically determined on micellar surfaces of protoplasm.

For this purpose the right-hand straight line part of the curve ob must be continued to the intersection with the ordinate axis. The sector od will numerically equal the adsorption limit A_∞. In the case discussed, the limit of the amount of sugar adsorbed by erythrocytes (A_∞), corresponding to the sector od, equalled 0.028 g per 55 g of dry residue of erythrocytes or per 100 ml of cellular water.

Another characteristic permeability constant—K (distribution coefficient of the substance between protoplasm water and the medium)—was also graphically determined.

129 The straight line oc parallel to db denotes the relationship between the amount of sugar dissolved in the protoplasm ($C_c - A_\infty$) and its concentration in the equilibrium liquid, which is in agreement with Henry's law (equation 2). Determining from the graph the value of C at a certain value of C_s from formula (2), the value of the distribution coefficient is found. In the case of galactose distribution between the surrounding water and the water of the erythrocytes, K = 0.42.

From the data in Table 25 an adsorption graph can be constructed, excluding the part of the substance which is dissolved. Figure 73 shows such a curve. It has the typical form of a Langmuir curve. The data calculated from the Langmuir formula are presented in the form of crosses, while those obtained experimentally are represented by circles. As is seen, the calculated and experimental data agree fairly well.

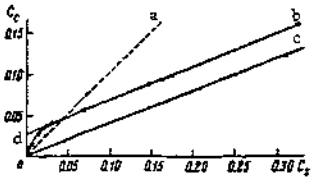


FIGURE 72. Relationship between galactose concentration in rabbit erythrocytes (C_c in g per 100 ml of intracellular water), and the concentration of the sugar in the equilibrium liquid of the medium (C_s, %). According to Troshin (1951b)

oa—bisectrix ($C_c = C_s$); ob—distribution curve; oc—relationship between the concentration of the fraction of the substance dissolved in the protoplasm, and its concentration in the medium; od—adsorption limit.

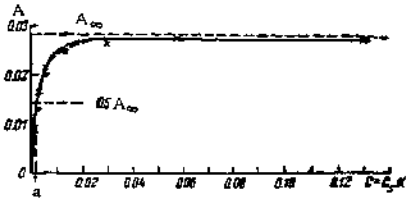


FIGURE 73. Adsorption isotherm of galactose by rabbit erythrocytes According to Troshin (1956)

The abscissa shows the concentration of galactose in the water of erythrocytes (in%); the ordinate shows the amount of the adsorbed galactose (in g per 55 g dry residue or per 100 ml cellular water). A_∞ —adsorption limit; a—the constant of the slope of the adsorption isotherm.

Table 25

The amount of adsorbed and dissolved galactose in rabbit erythrocytes (in g per 100 ml of water), at different concentrations of the sugar in the equilibrium liquid. According to Troshin (1951b)

Amount of water in erythrocytes (%)	Concentration of galactose		Fraction of total galactose present in erythrocytes		$Q = \frac{C_c}{C_s}$
	in medium (C_s)	in erythrocytes (C_c)	dissolved $C = C_s \times 0.42$	adsorbed $A = C_c - C$	
65	0.003	0.011	0.001	0.010	3.67
66	0.007	0.015	0.003	0.012	2.14
65	0.015	0.026	0.006	0.020	1.74
65	0.032	0.041	0.013	0.028	1.29
64	0.071	0.056	0.029	0.027	0.77
64	0.148	0.087	0.060	0.027	0.58
65	0.322	0.159	0.132	0.027	0.47

A number of authors point out the fact that with increase in the concentration of the sugar in the medium, its relative concentration in the erythrocytes decreases (Masing, 1914a; Ege, 1920c, 1921b; Hausler, 1925a, 1925b; Woodhaus and Pickworth, 1932, and others). The theory of Troshin gives a good explanation of these facts.

In general these were the main lines of thought which led Troshin (1951e) to divide the phenomena of permeability into two components—adsorption and solution. Using the same method for other objects and other substances Troshin showed that the distribution curve (Figure 72) is a graphic description of a very common rule, applying to nonelectrolytes and electrolytes. Examples of this will be given.

Figure 74 shows the distribution of 3 sugars (arabinose, galactose and saccharose) between striated muscles and the surrounding medium. For each of these curves, the same graphical analysis may be made as that performed for the distribution of galactose between erythrocytes and the medium (Figure 72). The intensity of adsorption (A_∞) on the protoplasm micelles of each of these substances, and the distribution coefficients, may be determined.

Arabinose was shown to be the most strongly adsorbed sugar ($A_\infty = 0.03$); galactose was somewhat less adsorbed ($A_\infty = 0.02$) and saccharose was adsorbed to the smallest extent ($A = 0.01$). These substances are arranged in the same order, also in relation to their ability to dissolve in the protoplasm of muscles. The highest distribution coefficient was that of arabinose (0.43), followed by galactose (0.32), and finally by saccharose (0.29). This series of sugars corresponds to that of increasing molecular weight.

The concensus of firm opinion in the literature is based mainly on osmotic experiments, according to which urea penetrates well into plant and
130 animal cells (Hamburger, 1889, 1891; Overton, 1895; Grijns, 1896; Hedin, 1898; Roncato, 1923; Mond and Hoffmann, 1928; Gellhorn, 1932; Hill, 1935; Wilbrandt, 1938a; Höber, 1945; and others). These data were usually treated from the point of view of the membrane theory. Meanwhile, many facts accumulated which did not agree too well with this theory. Many authors have observed that when urea penetrates into protoplasm it reaches a certain concentration level different from that in the environment. Such results were obtained by Wu (1922), Folin and Berglund (1922b), Lundsgaard and Holböll (1926), Folin and Swedberg (1930), Woodhaus and Pickworth (1932), Conway and Kane (1934), Parpart and Shull (1935a, 1935b), Williams and Kay (1945), and others. According to these authors' data, the intercellular concentration of urea was sometimes lower than the external one, sometimes equal to it, and in certain cases greater. The authors of these studies mostly used single concentrations of urea, sometimes chosen without sufficient foundation, probably assuming that the concentration itself should not affect the distribution coefficient between the protoplasm and the medium. In reality, this is not so. In the case of urea, as in the case of other substances, the equilibrium distribution coefficient (Q) varies, depending on the concentration employed*. This

---

* The coefficient (Q) should not be confused with the distribution coefficient K in Troshin's formula (3). The former is the distribution coefficient of the total substance, the adsorbed and the dissolved one: the latter is the distribution coefficient only of the dissolved fraction of the substance which has entered the cell.

was shown by Troshin using his ordinary method of finding equilibrium concentrations. Figure 75 B, C shows that from relatively weak solutions, muscle fibers take up urea at a concentration exceeding that of the surrounding medium. As far as stronger solutions are concerned, the concentrations inside and outside the cells are almost similar, the coefficient (Q) here approaching unity (Figure 75 A).

131

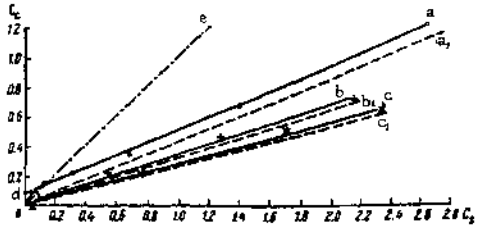


FIGURE 74. Relationship between the concentration of sugars in muscle fibers, (C_c) in g per 100 ml of intracellular water), and their concentration in the medium (C_s, in %) (according to Troshin, 1951e)

Sorption by muscle fibers: oa—of arabinose; ob—of galactose; oc—of saccharose. The amount of sugars present in the muscles in the dissolved state: oa_1—arabinose; ob_1—galactose; oc_1—saccharose.

Figure 76 illustrates the distribution of urea in the case of muscles. The first part of the curve ob is the same as in the case of other nonelectrolytes. Here adsorption prevails; therefore the concentration in the protoplasm is higher than in the medium. Later the curve approaches the bisectrix, and intersects it at the point c. By analogy with other substances, it might have been thought that further on, curve ob would acquire a straight linear course at a certain acute angle to the abscissa, corresponding to the solution of the urea in the protoplasm water. However, in the present case the ordinary course of the curve is markedly disturbed. The curve bends upwards, and intersecting the bisectrix for the second time (at point d), steeply rises. This is explained by the fact that concentrations of urea higher than 2% damage the protoplasm, causing denaturation (paranecrotic) changes. This fact does not permit exact determination of the coefficients K and A_∞ for urea.

The distribution of creatinine between human blood plasma and erythrocytes was studied by Wu (1922) and by Folin and Swedberg (1930). According to Wu, Q = 2. According to Folin and Swedberg, Q = 0.58-0.90.

In the case of muscles, the distribution of creatinine between the fibers and the medium was studied by Fenn (1936) and by Eggleton (1930). According to these authors' data, Q for creatinine is close to 1.

Troshin (1952) studied the distribution of creatinine in frog muscles in a large number of equilibrium concentrations. As always, the equilibrium concentration levels (Figure 77) were first determined for a number of solutions. Subsequently, on the basis of the data obtained, a distribution curve was plotted (Figure 78). In principle this curve did not differ from others obtained for the permeability of nonelectrolytes. At
132 first the muscles took up creatinine from weak solutions, to a concentration higher than the external one. This is the predominant region of adsorption. Subsequently, after adsorption saturation was complete, the intracellular concentration of creatinine increased in a rectilinear fashion at an acute angle to the absicssa. In this region solution of the substances in

protoplasmic water predominated, and the distribution of the substance obeyed the law of Henry. The acute angle of the slope of the straight line indicates that the solubility of creatinine in protoplasm water was lower than in the surrounding water. On the basis of these data, and by the use of the above-described method, both basic permeability constants—A_∞, K were determined. In case of penetration of creatinine into frog muscle A_∞ = 0.007 g per 100 g of intracellular water and K = 0.38. Consequently, solubility of creatinine in the sarcoplasm was 62% lower than in the water of the surrounding medium, independently of its concentration in the latter.

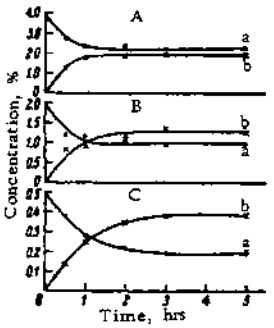


FIGURE 75. Uptake of urea by frog muscles from the surrounding solution (according to Troshin, 1954)

A,B,C- different initial concentrations of urea; a—disappearance of urea from the medium; b—uptake of urea by the muscles.

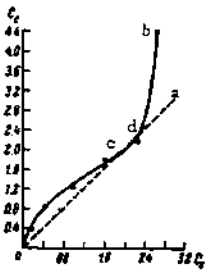


FIGURE 76. Relationship between the concentration of urea in muscle fibers of the frog (C_C, in g per 100 ml of intracellular water), and its concentration in the medium (C_S in %) under conditions of diffusion equilibrium (according to Troshin, 1954). For details see text.

It has already been mentioned that permeability of plant cells as determined by their plasmolysis is a fundamentally different phenomenon from the penetration of substances into nonvacuolated animal cells, as determined by direct chemical analysis*. In the former case the diffusion rate of the substance through a layer of protoplasm and tonoplast is studied, in the latter the rules of distribution of the dissolved substance between protoplasm and the medium. Undoubtedly a connection exists between these
133 two sets of phenomena, but this connection is not as simple as may seem at

---

* The problem of accumulation of substances in plant vacuoles is of special interest and will not be dealt with here. Those interested in this problem are referred to the book by Troshin, 1956.

at first sight. Only by experimenting with nonvacuolated plant cells can their permeability to be compared with animal cells, when the same method-direct chemical analysis- is applied.

The studies of Troshin (1948a) on permeability of yeast cells are of great interest in this respect. Before his studies such investigations were mainly performed by the use of the osmotic, gravimetric and volumetric methods (Swellengrebel, 1905; Paine, 1911; Sohngen and Wieringa, 1926; Rubinshtein and Verkhovskaya, 1935). These led to contradictory results. As seen from Figure 79, illustrating the establishment of equilibrium in the distribution of lactose between yeasts and the medium, the same rules of permeability are observed as with animal cells. A low concentration of lactose (2.3%) resulted in high intracellular concentration as compared with the surrounding solution (Figure 79, C). A certain intermediate strength of solution (8.1%) led to an approximate equilibrium of the concentrations inside and outside (Figure 79, B). Finally, in strong solutions (Figure 79 A), the concentration of lactose in yeast cells was lower than in the surrounding medium.

This is shown even more clearly by the distribution curve (Figure 80). Here the initial zone corresponding to the predominance of adsorption is quite extensive. Later, the curve crosses the bisectrix at a point where the external and internal concentrations are equal and passes into a region where the external equilibrium concentration predominates. In the given case the adsorption constant is very high ($A_\infty = 2.2$ g per 100 ml of water). The solubility constant $K = 0.55$. This means that in the proto-
134 plasm water of yeasts, solution of this nonelectrolyte is 45% lower than in the water of the surrounding medium.

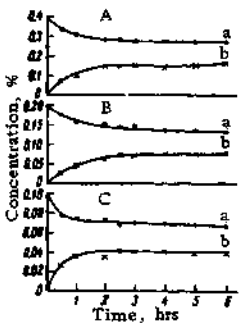


FIGURE 77. Entrance into frog muscles of creatinine from the surrounding medium (according to Troshin, 1952)

A, B, C-various initial concentrations of creatinine; a-decrease in creatinine in the medium; b-uptake of creatinine by muscles.

Figure 81 shows an adsorption isotherm of lactose in yeast protoplasm. This curve may be obtained by subtraction from each point of the distribution curve (Figure 80) of the corresponding concentration of dissolved lactose, as determined by the straight line oc. On this curve the circles designate the arithmetic means from a large number of experiments, while the crosses represent the points of the theoretical Langmuir curve for nonpolar adsorption according to the formula

$$A = A_\infty \frac{C}{C + a}$$

where A_∞ equals 2.2 and a equals 0.07. The figure illustrates well the agreement between the theoretical and the experimental data.

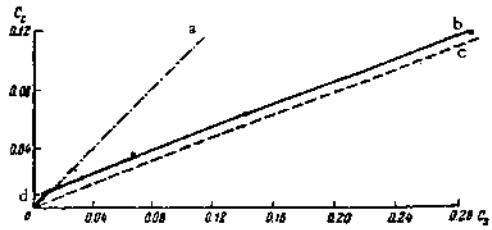


FIGURE 78. Relationship between creatinine concentration in muscle fibers of the frog (C_c, in grams per 100 ml of intracellular water), and its concentration in the surrounding equilibrium liquid (C_s, in percent) (according to Troshin, 1952)

Legend the same as in Figure 72.

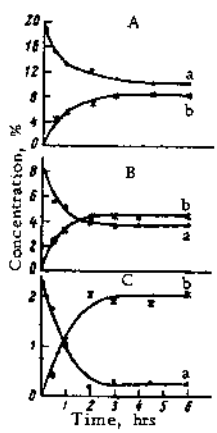


FIGURE 79. Uptake of lactose by yeasts (according to Troshin, 1951e)

A,B,C–various initial concentrations of lactose. a—concentration of lactose in medium; b—concentration of lactose in yeasts.

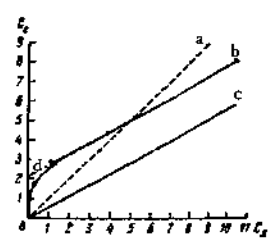


FIGURE 80. Relationship between lactose concentration in yeasts (C_c, in grams per 100 ml of intracellular water), and its concentration in the medium (C_s, in percent) (according to Troshin, 1951e). Legend the same as in Figure 72.

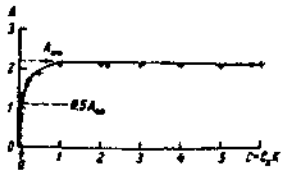


FIGURE 81. Isotherm of adsorption of lactose by yeast cells (according to Troshin, 1956)

Abscissa shows concentrations of lactose dissolved in yeast water (in percent); ordinate shows amount of adsorbed lactose (in g per ml intracellular water of per 48-49 g dry yeast residue. For other details see legend to Figure 73.

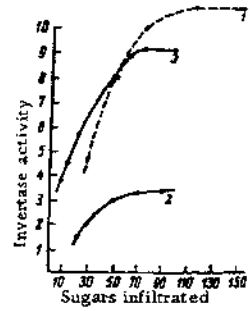


FIGURE 82. Relationship between (1) the rate of hydrolysis and (2, 3) synthesis (in mg of inverted sugar), and the concentration of infiltrated sugars (in mg per 1 g dry weight of leaf), in (1, 2), cyclamen leaves; (3) chicory leaves (according to Kursanov, 1936)

Thus, the example of permeability of yeast cells to a nonfermentable sugar—lactose—shows with special clarity the same physico-chemical distribution of nonelectrolytes between the cells and the medium. The essence of this has been shown to consist in the combination of two factors—(1) low solubility of the substances in the protoplasm water, and (2) their adsorption on micellar surfaces.

Evidently, adsorption is of greatest biological importance. Its main role is at relatively weak external concentrations of the substance, i.e., under conditions closest to physiological. With a higher concentration in the medium, adsorption ceases. Subsequently there is only an increase in the concentration of the substance dissolved in the protoplasm, which biologically is apparently less important. Troshin cites the following quite convincing considerations in favor of this point of view. He points out the well-known fact that the activity of enzymes depends to a certain extent on the concentration of the substrate. With increase in the substrate concen-
135 tration the velocity of the enzymatic reaction increases, but not without a certain limit. It reaches a certain level which it does not exceed. Curves of this type (Figure 82) are very similar to Langmuir's adsorption isotherms.

Many investigators assume that this similarity is based on the fact that heterogeneous catalysis, which plays an important role in metabolism, requires contact between the enzyme and the substrate. The similarity of these phenomena is illustrated by Troshin in an interesting diagram, in which he traced his curve of lactose adsorption to micellar surfaces of yeast cells protoplasm (Figure 83, 1), the activity of invertase (Figure 83, 2), and the rate of glucose fermentation (Figure 83, 3).

The correlation between these data cannot be accidental. It seems to us that it refutes the assumption often made by adherents of the membrane theory, that the method of direct chemical analysis supposedly detects substances which do not enter the cell, but are only adsorbed to its outer surface.

Distribution of Aminoacids between the Cells and the Medium

The study of permeability of cells to aminoacids is associated with one of the greatest paradoxes in this field of study. Aminoacids- substances absolutely essential for protein metabolism of cells- supposedly do not penetrate at all into the cells, according to many supporters of the membrane hypothesis. This opinion was held by Overton and Höber in relation to muscle fibers and this was also the opinion of a number of authors in relation to erythrocytes (Grijns, 1896; Kozawa, 1914; Hiruma, 1923 and others). At the same time, it has been established by the use of direct chemical analysis that in erythrocytes (Wu, 1922; Folin and Berglund, 1922; Zbarskii, 1925a; 1925b; 1936; Barkhash, 1934; Yampol'skaya, 1938; Kulakova, 1939; Friedland, 1939; Gur'eva, 1940; Saichuk, 1941; Demin, 1941a, 1941b; Hamilton and Van Slyke, 1943; Christiansen and Lynch, 1946 and others), and in fibers of skeletal muscles (Van Slyke and Meyer, 1913) and in other cellular elements (Miropol'skii, 1950), there is a higher concentration of aminoacids than in the surrounding medium. The distribution coefficient (Q) for erythrocytes fluctuates between 1.5 and 4.5, while for muscles it is from 5 to 10. Direct analysis of the content of the axoplasm of the giant nerve fibers of the cephalopod molluscs has shown that the concentration of aminoacids in it is several times higher than in their blood plasma (Schmitt, Bear and Silber, 1939; Silber and Schmitt, 1940, Silber, 1941, and others). The latter case is especially interesting since it completely excludes the possibility that aminoacids are only adsorbed to the cell surface (Höber, 1926).

Finally, it has been proven that the process of accumulation of aminoacids in the protoplasm may be a reversible one. This has been shown by Zbarskii and Zubkova (1934) with dog erythrocytes and by Saichuk (1941) with horse blood.

Troshin (1951d), studied in detail the distribution of alpha-alanine between the calf muscles of the frog and the surrounding fluid, by determining the established equilibrium concentrations of the substance. Parallel experiments were performed with muscles previously killed with 96° alcohol and thoroughly rinsed in Ringer's solution.

The process of establishing the diffusion equilibrium for alanine in experiments with living muscles is illustrated in Figure 84. As in the case of other substances studied, the equilibrium between the internal and external concentrations was reached after 1.5-2 hours, whereby the same rules seem to apply. At weak concentrations of alanine (Figure 84 B) the concentration level of this substance in the protoplasm was higher than the surrounding fluid. However, in the case of strong solutions (Figure 84 A) the intracellular concentration level lagged behind the external one.

Figure 85 describes penetration of alanine into killed muscles, and shows that there is no basic difference between the rate of penetration of alanine

into living and dead muscles. Nevertheless, there are basic differences in respect to establishment of equilibrium levels. In killed muscles, alanine penetrated as long as the intra and extra-cellular concentrations did not become equal. The picture resembles that for free diffusion in water.

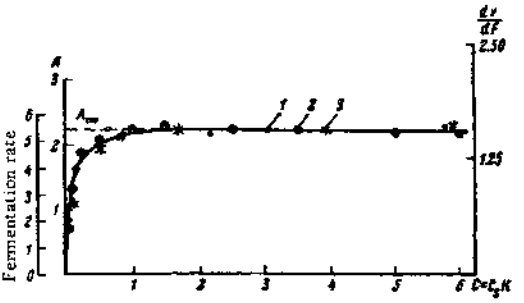


FIGURE 83. Adsorption isotherm for lactose by yeasts, and the relationship between the enzymatic activity and the concentration of the substrate in the medium.

The abscissa shows concentrations of lactose (in %) in the yeast water (or the corresponding concentration of sugars in the medium); the ordinate shows the amount of lactose adsorbed by the yeasts (A, in g per 48-49 dry weight of yeasts), the activity of invertase ($\frac{dV}{dF}$ according to Afanas'ev and Il'ina, 1949) and the rate of glucose fermentation (in ml, according to Slator, 1906)

1—adsorption of lactose; 2—activity of invertase; 3—rate of glucose fermentation (according to Troshin, 1956).

The distribution curve of alanine (Figure 86) does not basically differ from this type of curve for other substances. In the initial part the curve passes above the bisectrix, i.e. the equilibrium concentration in the protoplasm is higher than in the medium. This is the region of prevalence of adsorption. Subsequently the curve crosses the bisectrix and continues rectilinearly. This is the region of predominant solution. The constant, characterizing absorption in the case of permeability of alanine into muscles, $A_\infty = 0.029$ gm per 100 ml of intracellular water. For this substance $K = 0.40$. This means that there is 60% less free dissolved alanine in the water of muscle fibers than in the medium.

The distribution curve for alanine in dead muscles is entirely different (Figure 87, ob). It has the appearance of a straight line almost coinciding with the bisectrix (oa). Here, $A_\infty = 0$ and $K = 0.95$. This means that in

killed muscles alanine is not adsorbed by fixed protoplasm and its solubility in the protoplasm is only 5% lower than in the surrounding medium. This small decrease may be explained by the fact that 5% of the water in fixed muscles may be bound.

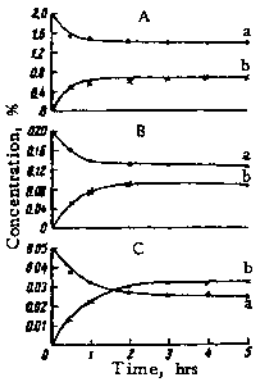


FIGURE 84. Entrance of alpha-alanine from the medium into muscle fibers of the frog (according to Troshin, 1951d)

A-C—different concentrations of alanine solutions. a—decrease in alanine in the medium. b—uptake of alanine by the muscles.

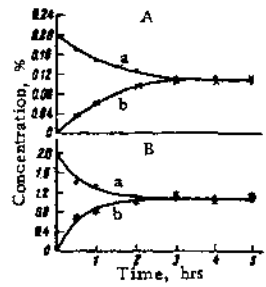


FIGURE 85. Entrance of alanine into killed frog muscles (according to Troshin, 1951d)

A, B—different initial concentrations of alanine. a—decrease of alanine in the medium; b—uptake of alanine by the muscles.

Thus according to Troshin's data the aminoacid alanine rapidly penetrates the muscles and reaches a considerable concentration there, especially when the uptake of this substance is from weak solutions. Ten years ago, these data might have been regarded as contrary to the then accepted theories of impermeability of cells to aminoacids. At present
38 however, these facts are in full agreement with a great number of studies recently performed by the use of labeled atoms*.

Protein turnover of the intact organism as determined by the introduction of radioactive amino acids, turned out to be astoundingly rapid. Thus, according to the data of Tarver and Reinhardt (1947), the aminoacid S^{35}—methionine, introduced into the blood of the dog was detected in all the internal organs after two hours, while after five hours only a very small additional increase in its content was observed. According to the

---

* For a review of the literature see papers by Schonheimer (1942), Konikova (1948), Borsook (1950), and others.

data of Borsook (1950), on introduction into rats of C^{14}—labeled aminoacids, an equilibrium between intra- and extra-cellular aminoacids was established after 30-60 minutes. Evidently, the labeled aminoacids entered the different organs at the same rate (Borsook, Deasy, Haagen-Smit, Keighley and Lowy, 1949).

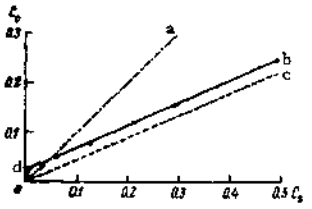


FIGURE 86. Relationship between the alanine concentration in muscle fibers (C_c) and its concentration in the equilibrium medium (C_s). (according to Troshin, 1951d). Legend the same as in Figure 72.

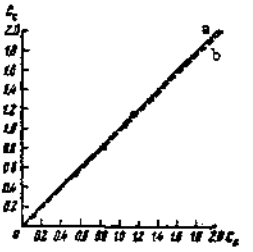


FIGURE 87. Relationship between alanine concentration in dead muscles (C_c) and its concentration in the surrounding equilibrium solution (C_s). (according to Troshin, 1951d). Legend the same as in Figure 72.

The above data show that all the cells are well permeable to aminoacids. Consequently, the belief that cells are not permeable to these substances is one of those erroneous ideas based on the faulty premise of the cell as an osmometer surrounded by a semipermeable membrane.

Distribution of Vital Stains between the Cells and the Environment

In the study of permeability special attention was always paid to the penetration of vital stains into the cells. The supporters of the membrane theory explained the greater or lesser penetration of stains into cells from the point of view of their diffusion through cell membranes. The ability of the more penetrating stains to better dissolve in lipoids usually served as one of the basic arguments in favor of the lipoid nature of the cell membranes (Höber, 1909, 1926, 1945; Gelhorn, 1932; Gutstein, 1932 and others).

However, there are many objections to this concept of the mechanism of penetration of dyes into cells. The existing data indicate that the basic rules of vital staining are connected with the phenomena of sorption of the dyes by protoplasm. Thus for example, only by sorption phenomena can the higher concentration of basic dyes inside cells be explained as compared

with the concentration in the medium (Q>1). Further, there are cases where under the action of different stimulants, considerable staining of the cell nucleus is seen at first and only later does the protoplasm staining become noticeable. Finally, the most convincing arguments against "membrane" explanations are the facts connected with the transition of vitally stained living cells from the stimulated condition back to the resting state. An example will be given which has already been mentioned in the previous chapter.

Two twin muscles were stained with neutral red, diluted not with Ringer's solution, but with distilled water which is a muscle stimulant. The muscles thus stained bind considerably more dye than muscles stained with a Ringer's solution of the dye. In terms of the membrane concept this is explained by the fact that distilled water damaged the membrane
139 ("loosened it up"), making it more permeable to the dye. In terms of our theory, the sorption properties of the proteins became stronger under the influence of the stimulant. The correctness of our explanation and the erroneousness of the "membrane" one is shown by the following experiment.

A pair of muscles was strongly stained in distilled water. One was submerged in pure Ringer's solution, while the other was placed in pure distilled water, both solutions without dye. The question is where the discoloration of the tissue would be quicker if the alteration caused by distilled water is reversible? According to the membrane theory, conditions which damage the membrane are maintained in distilled water, and the permeability of the membrane remains higher. Therefore the conditions will be more favorable for exit of the dye than in muscles placed in Ringer's solution. Here conditions are created for the restoration of low permeability of the membrane and diffusion of the dye that has entered the muscle fibers should be retarded by the membrane which regains its initial properties. It was easy to show experimentally that in distilled water, practically all the dye remained in the muscle, while in Ringer's solution, the dye rapidly left the muscle in the form of a stain cloud.

Obviously this result cannot be explained by decreasing or increasing the density of the membrane. Similar experiments can be performed after dye has left the cells, i.e., after restoring their normal sorption properties which were temporarily altered by a certain stimulant.

For us, the problem of penetration of dyes into cells is of special interest for a number of reasons. First of all, the intensity of vital sorption is an index of the physiological status of protoplasm. Stimulants may increase this sorption level considerably.

It is well known that the majority of dyes are completely dissociated macromolecular organic electrolytes. The basic dyes have a stained cation, while the acid ones have a stained anion. As will be seen later, the distribution of basic and acid dyes closely resembles the distribution of mineral cations and anions between the cells and their environment. A very important advantage of the vital staining method is that by its use, the distribution of the ions through the components of the cell can be studied.

Troshin (1951 c) used this method to study the distribution of basic and acid dyes in skeletal frog muscles. The following were studied: (1) neutral red (a basic dye causing weak granule formation in the sarcoplasm of muscles); (2) rhodamine (a basic dye staining protoplasm only diffusely) and (3) phenol red (an acid dye also causing diffuse vital staining of protoplasm).

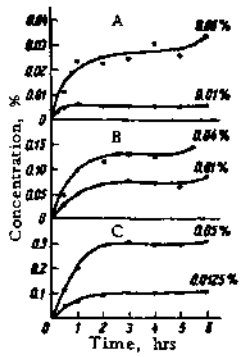


FIGURE 88. Uptake of vital stains from the medium by living sartorius muscles of the frog (according to Troshin, 1956)

A—phenol red; B—neutral red; C—rhodamine. The numbers above the curves designate concentrations of the dyes in the surrounding solution.

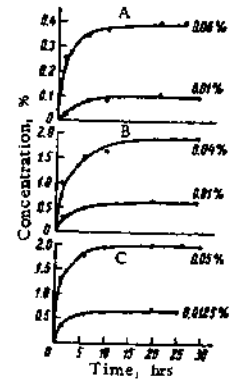


FIGURE 89. Uptake of vital stains from the medium by dead sartorius muscles of the frog (according to Troshin, 1956). Legend as in Figure 88.

140 The establishment of equilibrium between dye outside and inside the muscles was first studied on living and killed muscles, using different concentrations of the dye. The muscles were kept for different periods of time in solutions of the dye diluted with Ringer's solution, after which they were quickly wiped and dried on tissue paper and placed in acidified alcohol to extract the dye from the protoplasm. The resultant extract was measured colorimetrically in a Pulfrich photometer, and the result stated in terms of 100 ml of muscle water. The results of this series of experiments are given in Figures 88 (live muscles), and 89 (killed muscles). These figures show that the diffusion equilibrium was reached more rapidly with live muscles than with dead ones; in the former case, after 1-3 hours, and in the latter, after 5-10 hours. If permeability were assessed by the rate of establishment of diffusion equilibrium, the conclusion would thus be that permeability of living muscles is higher than that of dead ones, which is, of course, an utter paradox. In reality, dead muscles bind incomparably more dye than live muscles and transport of a much greater amount of dye into the cell is required in order to realize sorptional saturation, than in the case of living muscle fibers.

There is a notable difference in distribution of acid and basic dyes in living muscles. At equilibrium, there is approximately 2-2.5 times less phenol red in living muscles than in the medium, while the equilibrium concentration of neutral red and of rhodamine, on the other hand, is many times higher. This is explained by the fact that the proteins of living and killed protoplasm are negatively charged. The sorption of dyes takes place
141 either in the form of electro-adsorption upon which, first of all, the positively charged stained cations of basic dyes are adsorbed, or in the form of a simple exchange reaction between the mineral cations of the protein and the cations of the dye. In either case, the proteins should mainly bind the basic dye.

On the basis of data on establishment of equilibria (Figures 88 and 89), distribution curves of the dyes between the cells and the medium were drawn (Figure 90 and 91). Figure 90 shows such a graph for live muscles. On comparing this figure with those corresponding to the distribution of other substances (Figures 72, 74, 78, 80, and 86), it will be seen that there are no basic differences. In other cases two stages of distribution could always be distinguished. The first stage—distribution from weaker surrounding solutions—was characterized by prevalence of sorption. On the graphs, this was expressed by the location of the initial curvilinear section of the curve above the bisectrix. In the second stage, after the sorbing surfaces were saturated with the substance, the distribution was expressed by a straight line, passing below the bisectrix and corresponding to solution in the protoplasm according to the law of Henry. Both these stages are well seen in the distribution of nonelectrolytes between the medium and the protoplasm of erythrocytes, muscle fibers and yeast cells.

Figure 90 shows distribution curves of two basic dyes (oa and ob) which rise steeply considerably above the bisectrix (od) and the curve of the acid dye (oc) which is located almost totally below the bixectrix, in the form of a straight line at an acute angle to the abscissa. Basic dyes are so strongly absorbed by protoplasm that the saturation limit cannot be reached without damaging the living protoplasm. Therefore, the value of this limit cannot be graphically determined. Likewise, the distribution coefficient of the dissolved fraction of the basic stains could not be determined since their distribution curves were far from being rectilinear and did not cross the bisectrix. As far as acid dyes are concerned, their curves almost do not possess an initial curvilinear section and they are described in the form of a straight line (Figure 90, oc). This means that acid dyes are almost not sorbed by living protoplasm and their major part is present in the cells in a free dissolved state, their solubility in protoplasm water being very low. For phenol red the adsorption limit (A_∞), determined graphically, equals 0.001 g of dye per 100 ml of water, and the distribution coefficient (K) equals 0.25. Both these values for acid dyes are extremely low as compared with other substances studied.

Thus, Troshin has shown that vital stains penetrating living protoplasm, may exist there in two states—the sorbed and the dissolved one. We have already mentioned that in this instance, sorption means either electro-adsorption on micellar surfaces, or a chemical exchange reaction between the mineral ion of the protein and the stained ion of the dye of the same charge. In either case, the binding between the dye and the substrate is reversible.

There is also a third possible form of existence of the dye in protoplasm, i.e., the stable irreversible, or more correct, slightly reversible binding by the substrate. That is to say, the deposition of granules of basic and acid dyes in cells. It is known that having once appeared, the dye granules may exist in the cells for weeks and even months, as long as the cells live. On the death of the latter, the stained granules dissolve.

142 As will be seen later, the nature of distribution and binding of basic and acid dyes by living protoplasm closely resembles the distribution and binding of the mineral ions and cells. In both cases, there are three forms of existence of the substance in living protoplasm: free solution, easily reversible sorption, and slightly reversible stable binding.

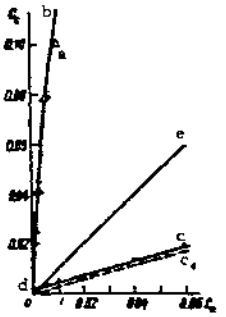


FIGURE 90. Relationship between the concentration of vital stains in muscle fibers (C_c in g per 100 ml of intracellular water), and their concentration in the medium (C_s, in %) at 19–20°C (according to Troshin, 1956)

a—neutral red; b—rhodamine; c—phenol red. For explanation see text.

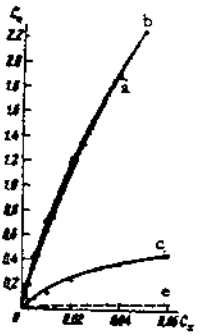


FIGURE 91. Relationship between the concentration of vital stains in killed frog muscles, and their concentration in the medium (according to Troshin, 1956).
Legend as in Figure 90.

Figure 91 shows distribution curves of basic and acid dyes on staining of killed muscles. The sorption of both by dead protoplasm increased to such an extent that it was impossible to draw the graph on the same scale, and the scale of concentrations in the cell on the ordinate had to be increased. Thus, the bisectrix od, which in Figure 90 indicated a similar distribution of the stain in the cell and in the medium, was locate in Figure 91 at a very acute angle. The curves of basic and acid stains are located considerably above the bisectrix. This increase in sorption as a result of killing the cells is not surprising. In the previous chapter, it was shown that increased sorption of dyes was characteristic not only of death of the protoplasm, but also of reversible damage inflicted on it. In our studies, as in Troshin's, increased sorption on death of the protoplasm was much stronger with acid stains than with basic ones. From this point of view, it is much more convenient to use acid dyes (phenol red) for the detection and quantitative study of paranecrosis.

143

Nonequilibrium of Electrolyte Composition of Cells and Environment

The interchange of electrolytes between cells and the surrounding medium is a central consideration in the theory of cellular permeability. It is specific for different types of cell, and changes markedly when the cell dies. The cation present in living protoplasm is mostly potassium, and the anions mostly phosphates. In the surrounding medium the predominant cation is

sodium, and the predominant anion chlorine. After death the cells lose potassium and phosphates, and gain sodium and chlorine. This process continues as long as a diffusion equilibrium is not established.

Table 26

Concentration of mineral ions in plasma, sciatic nerve, and muscles of frog (in m/moles per 100 gram of tissue or plasma: water in percent of wet weight of tissue). According to Fenn, 1936; Fenn, Cobb, Hegnauer and Marsh, 1934/1935; from Troshin, 1956

Ion	Plasma	Nerve	Muscle
Na^+	10.38	6.20	2.45
K^+	0.25	4.80	8.30
Ca^{++}	0.20	0.36	0.25
Mg^{++}	0.30	0.80	0.83
Cl^-	7.43	3.70	1.09
PO_4^{---}	0.31	1.00	1.28
HCO_3^-	2.54	1.08	1.07
H_2O	96.00	75.00	80.00

"Nonequilibrium in the distribution of electrolytes, as this phenomenon is usually termed, is found in the living state. The explanation of the problem is an old test of the theory of cell permeability, and its solution should provide a means of understanding one of the specific properties of living matter.

The explanation of bioelectrical phenomena is closely related to the problem of "nonequilibrium" in the distribution of ions in protoplasm, and consequently, a vital aspect of the contemporary study of physiology, i.e. electrophysiology, is related to it. The papers by Fenn (1936), Irving and Manery (1936), Kaplanskii (1938), Krogh (1946), Hodgkin (1951), Troshin (1956) and others give almost complete data on the distribution of ions between the protoplasm and the medium. It follows from these studies that the assymetric distribution of ions follows a very general law, probably without exceptions, and is applicable to the protoplasm of all multicellular and monocellular animals and plants. As an example, Table 26 gives analyses of the protoplasmic and plasma content of the more important cations and anions in nerves and muscles of the frog.

From the data given (Table 26) it will be seen that the mineral composition of the cell differs sharply from that of the surrounding fluid. The explanation of the mechanism maintaining this heterogeneity is of paramount interest to the physiologist.

The membrane theory in its original form solved this problem very simply. It was at first assumed that all the electrolytes detected in the living protoplasm by chemical analysis existed there in the form of a simple aqueous solution. It was also assumed that membranes of the majority of cells were permeable only to potassium ions, to cations equal or smaller in diameter (NH^+_4, H^+), and completely impermeable to anions. Membranes of erythrocytes, however, are totally impermeable to cations and semipermeable to anions. Under such conditions the electrolyte as a whole

144

(anion + cation) cannot diffuse out freely from the cell. This electrolytic "clogging" appears in protoplasm during growth and division of cells, as a result of increased metabolism. After being damaged, the membrane becomes permeable to all ions and the cell electrolytes pass freely to the outside, while the electrolytes of the medium entered the cell by diffusion.

However, this old and unfortunately prevalent concept also appears in comparatively new textbooks, and should now be recognized as completely erroneous. This error originally arose because previous authors drew their conclusions mainly on the basis of the indirect "osmotic" method. This was based on the concept of the cell as an osmometer surrounded by a semipermeable membrane. Recently, however, it has been established, mainly on the basis of direct data obtained by the method of labeled atoms, that both anions and cations freely enter the cells from the medium; however, the intercellular concentration never equals the latter.

Examples of this will be given in the three following chapters.

Permeability of Erythrocytes to Electrolytes

On the basis of experiments with osmosis, earlier authors maintained that the membranes of erythrocytes are completely impermeable to cations and only partially so to anions (Hamburger, 1891; Grijns, 1896; Hedin, 1897, 1898; Koeppe, 1897; Kozawa, 1914, and others). Permeability series were established for polyvalent anions on the basis of cell shrinkage in hypertonic solutions (Höber, 1945). The ready permeability of erythrocytes to many anions has often been confirmed by using labeled atoms (Hahn and Hevesy, 1942; Vladimirov, Pelishchenko and Urinson, 1947, 1948; Hevesy, 1950, 1951; Mueller and Hastings, 1951, and others).

However, many data were recently accumulated, on the basis of which permeability of erythrocytes, not only to anions but also to cations, may be considered to be a firmly established fact. Thus, Davson (1934) has shown that ox erythrocytes washed with potassium chloride are enriched with potassium at the expense of the sodium ion. The opposite may be observed in the presence of an excess of external sodium chloride. In a solution of glucose, sodium leaves the erythrocytes more rapidly than potassium. In the authors' opinion all these facts are difficult to understand in terms of the membrane theory. It is much easier to explain them by sorption mechanisms.

Numerous studies using labeled atoms support the concept of free penetration of potassium and sodium into erythrocytes. Such are the data of Hahn, Hevesy and Rebbe (1939a, 1939b), Cohn and Cohn (1939), Hevesy and Hahn (1941), Manery and Bale (1941) and others. It is also interesting that the results obtained by these authors in relation to the rate of penetration of sodium into erythrocytes, both in the organism and in vitro, was approximately the same. According to Hahn and Hevesy (1942), the rate of exchange of all the sodium of erythrocytes with labeled sodium of the
145 plasma is astounding. In rabbits this exchange occurs within 15 minutes, in dogs, in 40 minutes, and in cats, in 5 to 6 hours. According to their data, only a small part of the intercellular potassium is exchanged with potassium from the external environment; however, the rate of this exchange is higher than that of sodium.

Fenn and co-workers (Dean, Noonan, Haege and Fenn, 1941; Noonan, Fenn and Haege, 1941a; Mullins, Fenn, Noonan and Haege, 1941; Fenn, Noonan, Mullins and Haege, 1941) used labeled atoms and also observed the free penetration of radioactive potassium into erythrocytes, and its exchange with plasma potassium. The rate of this exchange differed in various animals. On the basis of their experiments, the authors reached the conclusion that the former concepts of physiologists on the impermeability of erythrocytes to cations were erroneous, and that it is impossible to explain the observed phenomena by the membrane theory. The same conclusion was reached by Hahn and Hevesy (1942), Hevesy (1950), and also by Kamen and Spiegelman (1948).

Finally, the works of Solomon and Gold (1952; Gold and Solomon, 1955) should be mentioned. By the use of radioactive isotopes they demonstrated the permeability of human erythrocytes to potassium and sodium. Likewise the group of studies by Maizels and Harris (Maizels, 1943, 1945, 1949, 1951, 1954; Feynn and Maizels, 1949; Harris and Maizels, 1951, 1952; Harris and Prankerd, 1953) performed by direct chemical analysis and by the use of radioactive isotopes with human erythrocytes and those of certain animals. These authors also established the permeability of erythrocytes to sodium and potassium and they showed that under any conditions deviating from the norm, potassium leaves the cell, while sodium enters it. After return of the erythrocytes to normal conditions, sodium diffuses out of the erythrocytes while potassium diffuses into them.

Permeability of Muscle Fibers to Electrolytes

Originally the proponents of the membrane theory assumed that the membrane of muscle fibers was impermeable to all anions, impermeable to sodium, lithium and calcium cations, and permeable to smaller cations, namely, potassium, rubidium, cesium, ammonium, and hydrogen. Thus, the selective permeability to ions was explained on the basis of the "sieve theory", the dimensions of the pores of this sieve being determined not only by their actual diameter but also by the charge of the membrane (Mond and Amson, 1928; Netter, 1928; Mond and Netter, 1930, 1932; Höber, 1936, 1945; and others). On the basis of these assumptions, an attempt was made to explain the asymmetry and distribution of ions in the fibers by the Donnan equilibrium (Netter, 1928; Mond and Netter, 1930, 1932; Höber, 1935). However, this explanation was completely refuted by the thorough experiments of Fenn and Cobb (1935a).

More recent studies performed by the use of direct chemical analyses, and in many cases by the use of radioactive isotopes, have shown that not only may potassium and ammonium freely enter muscle fibers and replace each other, although not in equivalent ratios (Fenn, Haege, Sheridan and Flick, 1944), but that sodium, too, may rapidly enter into the protoplasm and diffuse to the outside. It was originally assumed that all the sodium
146 and chlorine found in muscles is present not in the cells but in the intercellular spaces. However, this assumption was not confirmed. If this had been proved, then the ratio $\frac{Na}{Cl}$ in plasma would be the same as in muscles.

In reality this is not so. In man the ratio $\frac{Na}{Cl}$ is 1.37 in plasma, and 1.76 in muscles. According to the data of Fenn, Cobb and Marsh (1934), in the frog the ratio of $\frac{Na}{Cl}$ is 1.47 in plasma and 2.14 in muscles. Evidently, this part of the sodium is contained in the muscle fibers. However, these are only indirect fragments of evidence. In addition, there are also direct indications of sodium penetration into muscle fibers (Wu and Yang, 1931). The investigations of Kaplanskii and Boldyreva (1933, 1934) should also be mentioned. These authors showed that on breeding fish in a medium with a high sodium content, the concentration of this cation in the blood remains constant, while in the muscles its content increases from 51.5 to 83.4 mg percent. Kaplanskii and Boldyreva reached the conclusion that when sodium penetrates into the muscles, it forms a weakly dissociating compound with proteins.

A number of direct observations on the sodium penetration of muscles and its substitution for potassium were made with the use of radioactive isotopes (Heppel, 1939, 1940).

This investigator fed rats on food with a low potassium content, after which the potassium content in the blood of the animals decreased almost threefold, and in the muscles twofold. At the same time the sodium content of the muscles increased threefold. Heppel (1940) introduced labeled sodium into the peritoneum of these rats. Subsequently he estimated its penetration into muscles. The results were remarkable. All the sodium in the muscles was exchanged for labeled sodium ions from the plasma in less than 1 hour. Thus, the penetration of sodium into the muscles of warm-blooded animals is seen to occur very rapidly.

Keynes (1954) used radioactive isotopes and studied quantitatively the rate of interchange of potassium and sodium in isolated frog muscles. This rate, according to his data, was quite considerable (4.5 pikomoles per square centimeter of fiber area per second). The exit of potassium from muscles is somewhat faster. Increasing the concentration in the medium increases the rate of its penetration. Exit of sodium from the fibers occurs at the same rate as the penetration of potassium.

Penetration of radioactive sodium into muscle fibers was also confirmed by the studies of Manery and Bale (1941), Ussing (1947), Greese (1954) and others. This was also investigated in heart muscle by Krogh and co-workers (Krogh and Lindberg, 1944; Krogh, Lindberg and Schmidt-Nielsen, 1944). According to these authors the syncytial fibers of the isolated frog heart are permeable to potassium, sodium, calcium, glucose and saccharose. Depending on the concentration of ions in the medium, potassium and sodium may move freely in the direction of the syncytium or away from it, these movements occurring as if against a concentration gradient. The above authors being supporters of the membrane theory,
147 were forced to assume the existence of a certain mechanism within the membrane, which was able to pump ions in both directions with expenditure of energy. The presence of such a hypothetic "ionic pump" in the membrane was also assumed by Krogh (1946), Hodgkin (1951), Keynes and Maizel (1954) and others. However, Hodgkin emphasized that the real mechanism of "pumping off" of sodium from the protoplasm is still not clear.

It has already been mentioned that the original supporters of the membrane theory believed that no anions penetrate the membrane of non-stimulated muscle fibers (Höber, 1926; Mond and Amson, 1928; Rubinshtein, 1947). However, this basic assumption proved to be incorrect. If the chlorine ion indeed did not penetrate the fibers, and in the muscles was found only in the intercellular spaces, then the intercellular space determined by the chlorine present should be equal to that determined by the use of other anions, or with substances which do not penetrate the cell. In fact, this proved not to be so. According to the data of Boyle, Conway, Kane, and O'Reily (1941) the "chlorine intercellular space" of frog muscle comprised 14% of the total muscle, while the "magnesium" or "inulin" space comprised 9%. Clearly, a considerable part of the chlorine should be within the fibers. The above-mentioned authors were forced to come to this conclusion, although they were proponents of the membrane theory.

Fenn and co-workers (Fenn and Cobb, 1935a; Fenn, Cobb and Marsh, 1934) investigated in detail the "chlorine" and "sodium" spaces of muscles immersed in Ringer's solution. It was found that these spaces increased from 14.7 to 31% of the total muscle volume as the muscles were kept outside the organism. Originally these investigators assumed that chlorine was present exclusively in the intercellular spaces, and that these spaces actually increased when the muscles were kept in Ringer's solution. Subsequently, however, Fenn and Haege (1942) were forced to admit that chlorine partially penetrated the protoplasm of muscle fibers. Direct proof of the presence of chlorine in muscle fiber protoplasm was also obtained. Heilbrunn and Hamilton (1942) succeeded in analyzing isolated muscle fibers in frogs. Results showed that only 20% of the chlorine in the muscle appeared in the intercellular spaces, while 80% appeared in the muscle fibers. They calculated that the equilibrium concentration of chlorine in the fiber was 30% of its concentration in plasma. Results similar to these were obtained by Hess and Chu (1952) for isolated nerve fibers of vertebrates, and by Dean (1941a, 1941b) for muscle fibers. Steinbach (1937) demonstrated a high degree of permeability of Holothurioidea muscles to chlorine and potassium. Finally, Boyle and Conway (1941), Wilde (1945) and others reached the conclusion that chlorine may diffuse into the muscle fiber and leave it completely within a short time period if the muscle was immersed in a chlorine-free medium.

The main anion in the protoplasm of different cell elements is that of phosphoric acid. According to the supporters of the membrane theory, 148 compounds containing this anion exist in protoplasm in the dissolved state, and cannot diffuse out owing to the complete impermeability of the cell membrane to anions (Höber, 1926; Hill, 1930; Fenn, 1936; Boyle and Conway, 1941 and others). However, Embden and his co-workers (Embden and Adler, 1922) showed that phosphoric acid may leave isolated frog muscles at rest, but especially after excitation. These authors ascribed the exit of phosphates which they observed either to damage or to a state of excitation of the cell membranes. Fenn (1931, 1936) also observed the exit of phosphates from muscles, and he assumed that phosphorus left the cell in the form of some undissociated organic molecules. Exit of phosphorus from muscles was studied in greater detail by Il'in and Tikhal'skaya (1931), who arrived at somewhat different conclusions. According to their data, resting muscle cells are permeable to phosphorus but permeability

increases after excitation. They assumed that this increase was due not to increased membrane permeability, but to the formation of this anion de novo during muscle activity.

The high capacity of phosphoric acid compounds to penetrate muscle was recently demonstrated by Hevesy and Rebbe (1940). They introduced radioactive sodium and phosphorus into frogs, and after various time intervals determined their concentration in the calf muscles. After only 1 to 2 hours these anions reached approximately 20% of their concentration in plasma. With time the level of radioactive phosphorus increased, and after 3 days exceeded its plasma concentration 2.4 times. Thus, phosphorus not only entered protoplasm but its diffusion occurred as if against a concentration gradient. According to the authors' data, all the sodium present in the muscles was exchanged with plasma sodium during the first 2 hours.

Penetration of phosphorus into various organs in mammals was demonstrated by the same method of radioactive isotopes (Gaunt, Griffith and Irving (1941); Manery and Bale, 1941; Hevesy and Ottesen, 1943 and others). Furchgott and Shorr (1943) used this method to investigate phosphorus exchange in the heart muscle of a dog. They observed that the extra- and intracellular inorganic phosphates were exchanged without the mediation of organic phosphorus compounds. This observation is of special interest, since it is in opposition to the membrane theory, which postulated that phosphorus passed through the cell membrane only in the form of some organic compound (Fenn, 1936).

To sum up, the concepts of the membrane theory of permeability, according to which muscle fibers are impermeable to anions, were disproved by more recent studies, mainly with radioactive isotopes. It has been shown that muscles are permeable to all the cations and anions.

Permeability of Nerve Fibers to Electrolytes

The classical membrane theory considered nerve fibers, like muscle fibers, to be completely impermeable to anions and selectively permeable to cations. The same conclusion was drawn by Netter (1927), on the basis
149 of indirect data from the action of various salts on the resting currents in medullated nerves in frogs.

Later, Fenn and co-workers (Fenn, Cobb, Hegnauer and Marsh, 1934-1935) studied the electrolyte composition of the sciatic nerve in frogs. They compiled a table of interchange of ions between plasma and nerve fibers, based on an arbitrary assumption that the chlorine found was all in the intercellular space, to which unbelievably large dimensions had to be ascribed, i.e. 50% of the total volume of the nerve. This assumption was made in terms of the requirements of the membrane theory. Another aspect of this theory—impermeability to sodium—proved impractical since the "sodium space" exceeded considerably the dimensions of the "chlorine space". According to the data of Fenn et al. all the sodium and chlorine in a glucose solution rapidly left the nerve; one part of the potassium diffused to the outside and the other left the nerve only after its death. Thus, according to Fenn, sodium and potassium may freely penetrate nerve fibers.

Impermeability of nerve fibers to chlorine may be assumed, if it is implied that this ion is confined to the spaces between the fibers. It is very difficult to prove this in an intact nerve. However, this problem has recently been solved by direct chemical analysis of isolated giant nerve fibers of certain cephalopod mollusks. The possibility of impurities originating in the tissue fluid or in sea-water was excluded in these experiments.

On the basis of such analyses, Bear and Schmidt (1939) showed that the protoplasm of gigantic fibers of Tenthoidea contains potassium, sodium and chlorine, the sodium content being half that, and the chlorine a quarter that in the plasma. Thus, the presence of chlorine in protoplasm is beyond doubt, but a certain equilibrium concentration exists between the protoplasm and the surrounding fluid.

Steinbach (1940b; Steinbach and Speigelman, 1943) analyzed the axoplasm of the same tissue using the same method, and they found 369 millimoles potassium, 44 millimoles sodium, and 36 millimoles chlorine per kg body weight. After isolating the nerve and keeping it in sea-water, the amount of potassium in it decreased, while the chlorine and sodium increased. However, the content of these ions did not reach that in sea-water*. When the nerve was placed in isotonic glucose, all the sodium and chlorine left rapidly. After replacing the nerve in sea-water they again diffused into the fiber, reaching former concentrations. The authors concluded that nerve fibers of the squid are highly permeable to sodium and chlorine, and that the proved discrepancy in concentration of these ions outside and inside the fiber could in no way be explained by the "membrane" theory.

Permeability of nerve fibers of cephalopod mollusks to chlorine and sodium was confirmed by the work of Webb and Young (1940) and by Keynes
150 and Lewis (1959a). Keynes (1951b) studied the rate of penetration of radioactive isotopes of potassium and sodium into the giant fibers of cuttlefish. Results showed that potassium entered the fibers at a rate of 17, while sodium left at a rate of 61 pikomoles per second per cm^2 of the fiber area. The diffusion rate of potassium to the outside was 58 pikomoles, while that of sodium was 33 pikomoles.

The above data confirm the observation that nerve fibers are highly permeable to all cations and anions.

Electrolytes in the Protoplasm of Nonstimulated Cells

The above examples are sufficient to disprove the classical membrane theory. A rich collection of data has now accumulated, mainly by the method of labeled atoms. Analysis of the contents of isolated muscle and nerve fibers proves that all the ions can pass comparatively rapidly into

---

* Immediately after isolation there were 36 millimoles per liter of chlorine in the axoplasm. After 30 min in sea-water, this rose to 75 millimoles per liter, this concentration being maintained for a long time, notwithstanding the fact that the concentration of chlorine in sea-water is 540 millimoles per liter.

protoplasm and leave it for the outside. This has been demonstrated many times in in vitro experiments and in the intact organism.

Why then does the concentration of electrolytes outside and inside the cells not become equal? Why, while the cells lives, do not potassium and phosphates leave for the outside, where their concentration is much lower, and why do sodium and chlorine not enter the protoplasm? What forces keep them from moving in the direction of the concentration gradient? Why, in certain cases, for example during restoration after excitation or damage, do the ions begin to move as if against the concentration gradient, (potassium and phosphorus being directed into the cell and sodium and chlorine to the outside)?

On the basis of new data, we were convinced of the erroneous explanation of "nonequilibrium" by the membrane theory. As we have seen, certain investigators, in order to explain the nonequilibrium of ionic composition of protoplasm, and still retaining the old concept that intracellular electrolytes all exist in a dissolved, free condition, proposed a new hypothesis. According to this, the cell membrane supposedly possesses a certain mechanism which continuously pumps out sodium ions from the cells, due to which their concentration at any given time is lower in protoplasm than outside it. This creates conditions for the concentration of other ions in the cells, due to the Donnan equilibrium. It is self-evident that the work of such a hypothetic "sodium pump" (Hodgkin, 1951), necessitates a constant expenditure of energy at the expense of intracellular metabolism.

As a pure speculation, this hypothesis would be acceptable. However, to us it seems improbable. If such a mechanism existed, why then should it be connected with the cell membrane which, according to the membrane theory, should not exceed a few molecular layers in thickness? The pumping off mechanism could also be localized within the protoplasm.

Recent literature indicates that many known facts are incompatible with the theory of the "sodium pump" (Shaw and Simon, 1955; Troshin, 1956). The theory requires that all the potassium and sodium in protoplasm exist in the form of a free solution. It will be shown later that this requirement contradicts many contemporary studies which indicate that a
151 considerable fraction of the cell potassium (60-80%), and a part of the sodium, is chemically bound by protoplasm and is not ionized. Troshin (1957) and Troshin and Pisareva (1958), elicited data which are especially convincing in this respect, by determination of specific radioactivity. They showed that only a small fraction of potassium, and a certain part of the cell sodium, may be freely exchanged by radioactive isotopes. The remaining potassium and sodium is not exchanged for long periods of time. Thus the "pump" theory would be acceptable only after assuming that this "pump" is capable of selectively distinguishing labeled elements from unlabeled ones. This is completely out of the question. In our opinion, these facts completely refute the theory of the "sodium pump".

An earlier explanation (Nasonov and Aleksandrov, 1943a) seems to us much more probable. This was that the membrane theory was based on the assumption that all the protoplasmic electrolytes existed in a free dissolved state. This assumption is vitally important to the membrane theory in order to explain the equality of osmotic pressures inside and outside the cell. Many recent analyses indicate that a considerable proportion of

the electrolytes in living protoplasm (mostly potassium and phosphates) exist there not as a simple aqueous solution, but firmly bound to proteins. These are not ionized, and are not capable of free diffusion. In addition, protoplasm is a less effective solvent of several nonelectrolytes than the water surrounding it. The same is true of electrolytes. Therefore the dissolved fraction of electrolytes is in equilibrium with their more concentrated solution which surround the cell.

These are the two factors which cannot be ignored while attempts are made to explain the nonequilibrium of ionic composition of protoplasm, and which actually make it possible to explain this phenomenon. There is no need, therefore, to rely on the hypothesis of the "sodium pump", which to our mind, is not a very probable one.

The following are the important points supporting the theory that part of the mineral substance is bound to protoplasmic proteins.

1) Deficiency of anions. On estimating the mineral composition of protoplasm, all investigators have found a great predominance of cations over anions. Thus, it has been observed that 17% of the cations found in human erythrocytes are not compensated for by mineral anions. It has been assumed that this excess of cations is bound to protein anions (Kramer and Tisdall, 1922).

According to the data of Page (1927) the number of cations in unfertilized sea-urchin eggs is 6 times greater than the equivalent amount of anions.

A considerable prevalence of cations over anions in frog muscles was detected by Fenn (1936, 1938a) and also by Hill and Kupalov (1930). In the sciatic nerve of frog, Fenn et al. (Fenn, Cobb, Hegnauer and Marsh, 1934) found that the amount of cations was twice as great as the equivalent amount of anions. The deficiency of anions was detected by Bear and Schmitt (1939) in the axoplasm of giant nerve fibers of the squid Tenthoidea. According to their data the excess of cations in this tissue is not covered by the anions Cl , HCO_3 , OH , SO_4 and HPO_4 present.

152 Finally the work of Epshtein (1947) must be mentioned. He observed that in the brain tissue of rats, inorganic bases were seven times greater than the amount of known inorganic acids. In the author's opinion the excess of cations was bound to lipoids, proteins, aminoacids and other substances.

All these data, however, may serve only as an indirect indication of the possible bond between cations and proteins, since many authors suspect the presence of certain still unknown acid residues in protoplasm. The nature of the bonds themselves is also unknown. Ionized cations should be exchanged with excess cations in protoplasm. As a result, equilibrium between intra- and extracellular sodium and potassium should be attained. However, the prevalence of cations in the cells is sometimes so great that the excess obviously cannot be bound to protoplasmic proteins. In such cases firmer bonds would have to exist.

2) Binding of proteins excreted from protoplasm, to potassium, calcium and magnesium. It is now an established fact that when certain conditions prevail, the proteins excreted from the protoplasm contain chemically bound potassium. The first to draw attention to this fact were Neuschlosz et al.(1923, 1925a-1926c; Neuschlosz and Trelles, 1924; Neuschlosz and Walter, 1926). There have been several justified objections

to the method used by these authors (Simon and Szelöczey, 1928; Gallison, 1931 and others). Subsequently, however, many investigators, using a meticulous technic, have shown that a considerable part of the cell potassium is really protein-bound and nonionized (Ernst and Fricker, 1934a).

Frozen frog muscles were ground, and acetone extracts prepared; 1/3-1/5 of the total cell potassium passed into the filtrate, the remaining potassium being firmly bound to the protein residue. These data were completely confirmed in the works of Reginster (1937), who used a similar method and showed that four times more bound potassium is found in the muscles than free potassium. Similar results were obtained by Kometiani et al.(Kometiani, Dolidze and Klein, 1944; Kometiani, Klein, and Dolidze, 1946; Kometiani, 1947, 1948a, 1949). According to their data, 70-85% of the potassium exists in muscles in a form partly bound to proteins (myogen, myoalbumin, and globulin X) and partly in a nonionized state. The binding of cell potassium by protoplasmic proteins was confirmed by Szent Györgyi (1947), who used his results to establish the theory of muscle contraction. It has been shown that the myosin excreted from the muscles may be reversibly bound to potassium, calcium and magnesium.

It is of special interest to us that in the experiments performed in Szent Györgyi's laboratory, potassium split off from the protein and passed into solution after denaturation of a myosin solution with alcohol in the presence of anions. When the myosin remained for 48 hours at 0° C, half of the bound potassium was released and passed into the surrounding solution.

Mullins (1942a) showed that purified myosin gel, placed in a solution containing sodium and potassium in the same concentrations, took up and bound almost 4 times the amount of potassium in the medium, while sodium
153 was evenly distributed. Myosin denaturated at a temperature higher than 37° C lost this ability to absorb potassium selectively.

According to the data of Tarusova and Burlakova (1939), potassium did not leave the cells after hemolysis of erythrocytes with distilled water, while on hemolysis with saponin, it diffused outside. It is self-evident that these data exclude the possibility of intracellular potassium existing in a free state and being retained by the membrane. The authors themselves, as well as Wilde (1945) and Steinbach (1940a, 1940b, 1944), arrived at the same conclusion. The latter assumed that approximately 50% of the protoplasmic potassium was bound to proteins and was osmotically inactive.

Finally there are indications that a considerable part of the Ca and Mg in the cells is also bound to protoplasmic proteins (Dubuisson, 1942; Berwick, 1951). According to Berwick, part of the bound calcium passed into solution after the action of ether, cocaine and chloroform.

3) Determination of specific radioactivity. This method merits special attention since the data were obtained with intact animals, the normal functions of which were in no way disturbed. The method was as follows: potassium chloride, or another compound containing this element at a known specific radioactivity, was introduced into the blood of the animal. The specific radioactivity of various organs was determined at different time intervals, and from the results it was concluded what fraction of the potassium of the given organ was substituted by potassium from without. In complete agreement with data from proteins extracted from tissues, it was shown (in the intact organism), that only a small part of the cell potassium was capable of being exchanged for serum potassium.

Probably another part was firmly bound to protoplasm, was not ionized and could not be exchanged. By the use of this method Mullins, Fenn, Noonane and Haege (1941) showed that in frog, rat, and dog erythrocytes 20% of the potassium may be exchanged for radioactive serum potassium. In guinea pig, rabbit, and ox erythrocytes 40% of the potassium may be exchanged, and only in cat erythrocytes was all the potassium exchanged. This was probably due to the fact that the latter are almost devoid of potassium.

Hevesy and Hahn, 1941; Hahn and Hevesy, 1941a, 1941b, 1942, using this method, studied the potassium exchange of skeletal muscles, erythrocytes, brain and liver. According to their data, about 60-70% of the intracellular potassium was bound and not exchanged. However, all the sodium and chlorine may rapidly be exchanged with the medium. Troshin (1956), studying exchange of muscle potassium with radioactive potassium (K^{12}) of the medium (the results of which are described in Figure 92), confirmed this observation.

The amount of potassium in muscle protoplasm which took part in the exchange comprised only 30% of the total. Consequently, 70% of muscle potassium was bound and not capable of exchange.

In our laboratory Troshin (1957) and Pisareva (Troshin and Pisareva, 1958) studied in detail the state of potassium and sodium in frog muscles. Their results confirmed that the original acceptance of the "sodium pump" hypothesis was wrong.

154 In their experiments they placed isolated sartorius muscles of frog in Ringer's solution for various time periods. Subsequently, by a chemical method, they determined the changes in potassium and sodium concentration. The rate of exchange of potassium and sodium in the muscles with corresponding labeled cations from Ringer's solution was measured under the same conditions using radioactive Na^{24} and K^{42}. It was found that normal muscle (before immersion in Ringer's solution) contained 2.29 ± 0.17 m equiv. of sodium per 100 g of wet weight of tissue; after 10 minutes in Ringer's solution, the muscle contained 2.30 ± 0.34; after 30 minutes– 2.67 ± 0.25; after 2 hours– 3.38 ± 0.33; after 3 hours– 3.53 ± 0.55; and after 5 hours– 3.85 ± 0.55 m equiv. percent. The concentration of potassium in muscles kept under the same conditions was substantially normal (8.7 m equiv. percent) during the first day, and only toward the end of the second day decreased to 5.56 m equiv. percent. The amount of water in muscles also remained constant at 79–81%.

Later, while studying the change in radioactivity of muscles kept for different periods in Ringer's solutions containing Na^{24} or K^{42}, Troshin and Pisareva showed that both potassium and sodium were present in muscles in three fractions, which differed sharply from each other in their rates of exchange with the medium. They showed that the exchange reaction of muscle sodium and potassium with labeled sodium and potassium of Ringer's solution, respectively, obeyed the following equation:

$$C_t = ae^{-k_1 t} + be^{-k_2 t} + ce^{-k_3 t},$$

where C_t is the concentration of the unexchanged sodium or potassium in time t; a, b, and c are the initial concentrations of sodium or potassium in muscles ($t = 0$) of the first, second and third fractions respectively; k_1, k_2, and k_3 are rate constants of sodium or potassium exchange for fractions a, b, and c respectively.

After $2\frac{1}{2}$ hours in Ringer's solution the concentration of sodium in the muscles was 3.5 m equiv. (C_0 at t = 0), a—1.05 (30%), b—1.75 (50%), and c—0.70 (20%) m equiv per 100 g of wet weight of tissue, and the rate constants of exchange were (in hour^{-1}): k_1—23.8; k_2—3.89; k_3—0.032. It followed that the sodium in fraction c was exchanged with labeled sodium in Ringer's solution at a rate approximately 700 times lower than the sodium in fraction a, and 100 times lower than the sodium in fraction b.

On the basis of the above data, Troshin and Pisareva concluded that fraction a constituted the sodium of the intercellular spaces, fraction b the sodium of muscle fibers, and fraction c—bound (nonionized) sodium. The calculation, from the sodium of fraction a, of the dimensions of the intercellular space of the sartorius muscle, gave a result of 9.5% of the
155 total volume of muscle. The dimensions of this space, as estimated by other methods ("inulin space", "sulfate space", etc.) by a number of authors, gave a value close to this—9 to 12%. Later Troshin (1957), showed that increased sodium concentration in muscles, when the latter were kept in Ringer's solution, was due to increase in the free sodium fraction (fraction b). Under conditions of diffusion equilibrium the ratio between the sodium concentration in this fraction to that in the medium (K) equalled 0.31. Within a wide range of sodium concentration in the medium, the coefficient K did not change, i.e., the concentration of sodium dissolved in the water of the muscle fiber (C, fraction b) changed depending on its concentration in the medium (C_3) according to Henry's law: $C = C_3K$. The same value of K for sodium and chlorine was obtained by Troshin (1956) by the use of a chemical method.

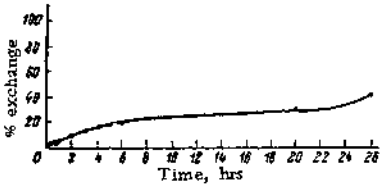


FIGURE 92. Exchange of potassium in sartorius frog muscles with potassium from Ringer's solution (according to Troshin, 1956)

Troshin and Pisareva obtained the following constant values for potassium in the above equation. At the beginning the concentration of potassium in muscles C_0 (at t = 0) equalled 8.7 m equiv. a was 2.0 (3.1%), b was 1.3 (15.6%) and c was 7.2 (81.3%) m equiv. per 100 g of wet weight of tissue. The rate constants for muscle potassium exchange in the corresponding fractions are (in hour^{-1}): k_1—4.4, k_2—0.24, k_3—0.02. In other words fraction a exchanged at rate 220 times faster than fraction c and approximately 20 times faster than fraction b. It may be assumed that the potassium of fraction a was that of the intercellular spaces. However, if it is taken into account that the dimensions of these spaces are approximately 10% of the muscle volume, knowing the concentration of potassium in the medium, it may be calculated that in the intercellular spaces about 0.4 m equiv. of potassium was found. The remaining part of the potassium in fraction a (0.16 m equiv.) comprised dissolved (free) potassium of the muscle fibers. The fraction of the muscle potassium b was in all probability labile bound potassium (adsorbed), while fraction c was stable chemically bound potassium. The same distribution of muscle potassium in fractions, and the dimensions of these fractions, were earlier found by Troshin (1956) on the basis of appropriate treatment (by other methods) of the data of certain authors. As already stated, these data completely contradict the "pump" theory.

Hodgkin (1951) and Keynes and Lewis (1951a, 1951b; Keynes, 1951a, 1951b) reached different conclusions. In their opinion all the potassium in protoplasm exists in the free state. It is highly probable that this contradiction was due to the fact that the authors studied the specific radioactivity of isolated giant nerve fibers of cephalopod mollusks (Keynes, 1951a, 1951b) and of isolated bundles of nerve fibers of the crab (Keynes and Lewis, 1951a, 1951b). These two tissues survive for comparatively short periods of time in artificial saline medium. According to the available data, their excitability, and the magnitude of the peak, decreased considerably after 20 minutes in these media (Prosser and Chambers, 1938), but under the experimental conditions employed, the fibers of Sepia (cuttlefish) had to be kept in this medium up to 4 hours, while crab fibers were kept up to 15 hours. It is possible that during this time the protoplasm of the fibers became damaged.

As stated, the total intracellular concentration of phosphorus is considerably higher than in the surrounding medium. According to Fenn (1936) the total phosphorus concentration in muscles is ten times greater than that of serum. The major part of this phosphorus enters into the composition of organic compounds (nucleoproteins, lipids, and others) and is thus
156 fixed in protoplasm. However, inorganic phosphorus is partially bound. Experiments performed by the determination of specific radioactivity of phosphorus in the medium and in cells confirm this hypothesis. A great many studies using this method demonstrate that in a number of tissues and cells (erythrocytes, muscles, yeast cells and others) after equilibrium has been established, the specific radioactivity of inorganic phosphates of the medium is higher than that of the inorganic phosphates excreted from cell protoplasm (Hevesy a. Aten, 1939; Hahn a. Hevesy, 1942; Mullins, 1942b; Furchgott a. Shorr, 1943; Juni, Kamen, Spiegelman a. Wiame, 1947; Mueller a. Hastings, 1951; Eichler u. Schmeiser, 1951; and others). These data were differently interpreted by different investigators. Some of them (Sacks, 1944, 1948; Kamen a. Spiegelman, 1948; Kamen, 1949; Lindberg, 1950; Eichler u. Schmeiser, 1951, and others) maintain that orthophosphate, as such, does not penetrate the cells, but is esterified at the surface of the cell membrane, and only subsequently enters the protoplasm. With regard to cellular orthophosphate, this is supposedly formed in protoplasm as a result of the decomposition of organic compounds. Contrary to this opinion, other authors assert that orthophosphates may penetrate the cells directly (Elliott and Hevesy, 1950; Brooks and Chambers, 1954). Chambers and White (1954), studying the accumulation of phosphates in sea urchin eggs, reached the conclusion that the inorganic phosphate found on analysis of protoplasm was an artifact originating from decomposition of some labile organic compound during its extraction from the cells. According to Soreni and Chepinoga (1946), Kometiani (1948a, 1948b), Velick (1949) and Klotz and Urquhart (1949), orthophosphate and other anions may be somehow bound to proteins excreted from the cells, which would seem to confirm the observation.

4) Leaching of mineral substances from tissues. Numerous experiments with washing of potassium out of cut tissues by potassium-free solutions, and also perfusion of whole organs by similar solutions, confirmed the hypothesis that a considerable part of cell potassium is bound. Many studies have been devoted to the investigation of the speed and sequence of exit of electrolytes from isolated muscles and nerves submerged in isotonic

glucose (Urano, 1908; Fahr, 1909; Fenn, Cobb, Hegnauer and Marsh, 1934/1935; Boyle, Conway and others, 1941). Thus, Fenn and co-workers (Fenn, Cobb, Hegnauer and Marsh, 1934/1935) have shown that sodium, chlorine and part of the potassium were the first to leave a frog nerve submerged in a 4.5% solution of dextrose. The balance of potassium only left after the death of the cells (Figure 93). The same results were obtained by authors using single giant nerve fibers of cephalopod mollusks (Steinbach, 1941; Steinbach and Spiegelman, 1943, and others). Their data are especially convincing, since in their studies the possibility of electrolytes being washed out of the intercellular spaces was excluded.

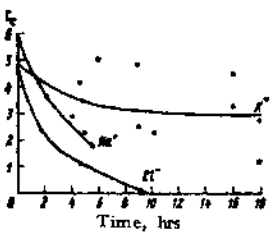


FIGURE 93. Decrease in the amount of potassium, sodium, and chlorine ions in frog nerve kept in a 4.5% dextrose solution (C_c, in m equiv per 100 g of nerve) (according to Fenn, Cobb, Hegnauer and Marsh, 1934)

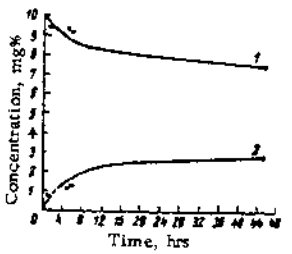


FIGURE 94. Decrease in calcium in frog calf muscles kept in calcium-free Ringer's solution, (according to Troshin, 1956)

1—decrease in calcium in muscles; 2—increase in calcium concentration in Ringer's solution.

Similar results were obtained by Ernst et al. (Ernst and Schaffer, 1928; Ernst and Takacs, 1931) who perfused potassium-free Ringer's solution, or isotonic saccharose, through frog muscles. In the former case, only a fraction of the potassium passed into the perfusate, while the main part left the muscle only after its death. Sodium, chlorine and a fraction of potassium readily entered the sugar solution. The major part of potassium and phosphorus, however, did not leave the muscles as long as these were not damaged.

157 Regarding calcium, it may be stated, a priori, that not all of it may exist in the cell in the free state if it is assumed that phosphates are also free in the cell, because in the presence of the latter, calcium would form a precipitate. There are, however, direct indications that a considerable part of calcium is bound to the substrate.

Figure 94 illustrates the exit of calcium from frog muscles into a calcium-free medium. Since the amount of liquid, as compared with the volume of the muscles, was small under the conditions of this experiment (an amount equal to the volume of the muscles themselves), an increase in the medium was observed simultaneously with the exit of calcium from the muscles. This process was observed for 8 hours, after which time no more

calcium left the muscles, and the intercellular level remained constant for 48 hours. Obviously, the remaining calcium was somehow bound to the substrate. In addition, it was seen that the calcium concentration in protoplasm was almost three times greater than its concentration in the surrounding Ringer's solution.

State of Electrolytes in Protoplasm after Excitation

The above data all indicate that in the living, undamaged, and non-excited cell, only a small part of the mineral substances is in the free dissolved state, while the major part is bound to protoplasmic proteins and is not ionized. This bond, however, may be rapidly severed due either to noxious stimuli, or after physiological excitation, the released electrolytes diffusing out. The reversible decrease in the muscle volume after excitation, discovered by Ernst (1928), is an indirect proof of this. In the author's opinion, this fact may only be explained by the appearance in the protoplasm of stimulated cells of free ions surrounded by solvation layers which, as is
158 well known, are of greater density than that of water. Later, Ernst and Fricker (1934a) showed that 2 to 3 times more potassium entered acetone extracts of muscle "snow" prepared from muscles in contraction, than extracts from resting muscles. These facts were confirmed by some highly convincing studies of Reginster (1937). According to his data the amount of bound potassium decreased twofold in muscles excited directly or through the nerve.

The exit of potassium from, and the entry of chlorine and sodium into muscles following direct or indirect excitation (through the nerve) has been described by many investigators, who found this reaction to be freely reversible. After excitation, potassium again enters the muscles, as if against a concentration gradient, while chlorine and sodium leave, also moving in the direction of a more concentrated solution. These phenomena were observed in cold-blooded and in warm-blooded animals (Ernst u. Schaffer, 1928; Ernst u. Csúcs, 1929; Mond u. Netter, 1930; Ernst u. Fricker, 1934a, 1934b; Netter, 1934; Fenn, 1935, 1937a, 1937b, 1938b, 1939; Fenn and Cobb, 1935a, 1935b; Fenn, Cobb, Manery and Bloor, 1938; Heppel, 1940; Noonan, Fenn and Haege, 1941a, 1941b; Wood, Collins and Mos, 1939/1940, and others).

Similar phenomena were observed on stimulation of nerves. Here, as in the case of muscle fibers, potassium began to leave the protoplasm of nerve fibers (Cowan, 1934; Young, 1938; Arnett and Wilde, 1941; Hodgkin and Huxley, 1947, 1953; Keynes, 1951b).

The exit of potassium and phosphates from cells after excitation is usually regarded, in terms of the membrane theory, as a result of increased permeability of cell membranes. This concept became firmly rooted in physiology and seems to conform to the appearance of electronegativity on the surface of the activated cell. According to our theory, the phenomenon is not one of increased permeability of the surface membrane, but of release of potassium and phosphates bound to protoplasmic proteins. This concept became the cornerstone of the theory of bioelectric phenomena (Nasonov and Aleksandrov, 1940, 1943a and 1944). As can be seen from the above data, this theory was considerably reinforced during the last two

decades, while the membrane concept of excitation (in its previous form) became utterly unacceptable.

The major difficulty of the membrane theory was to explain the return of potassium and phosphate into resting protoplasm, after having left it during excitation. Indeed, if the reason for the exit of electrolytes was the increase in permeability of the cell membrane on excitation, then after cessation of the stimulus the membrane should regain its former properties and again become impermeable to anions and to some of the cations. In that case it is difficult to understand which forces cause the electrolytes to reenter the cell after having left it. The well-known specialist in the field of protoplasm electrolytes—Fenn, a supporter of the membrane theory, was in this case also forced to admit that this theory "serves in fact, as a veil behind which we hide our ignorance" (Fenn, 1936, p. 482).

These difficulties do not arise if the sorption theory of permeability is accepted. In the resting cell most of the potassium and phosphorus is bound to proteins. After damage or excitation, the proteins lose their ability to bind these ions* and the latter diffuse freely to the outside. After
159 cessation of the stimulus the protoplasmic proteins regain their ability to bind potassium and phosphates. The intercellular concentration of these substances therefore decreases sharply, creating conditions for their diffusion from the medium into the cell. There is no interchange against a concentration gradient in this case, since the concentration of free potassium and phosphates in the protoplasm is very small and may even be lower than in the surrounding medium.

It has been shown by many investigators that after excitation, diffusion of sodium and chlorine into the protoplasm occurs simultaneously with exit of potassium and phosphates from the cell. This process is reversible.

How can this phenomenon be understood in terms of the sorption theory?

It will be seen later that chlorine and sodium ions are 2-3 times less soluble in living, resting protoplasm, than in the surrounding water. The solubility ratio is thus approximately 0.30-0.45. At the same time, sodium and chlorine are almost completely soluble in the protoplasm. It is known that following the action of irritants, and after death, protoplasm loses its phase properties and the substances begin to dissolve in the water contained in it as they do in the water of the surrounding medium. There are good reasons to believe that the same phenomenon occurs after excitation. The capacity of protoplasm to dissolve ions increases (K increases from 0.3 to 1.0), as a result of which Na^+ and Cl^- move in the direction of the cell. After excitation, the original properties of protoplasm are restored, including the property of being a poor solvent, and sodium, together with chlorine, then diffuses out.

The physiological meaning of increased membrane permeability after excitation, as postulated by the membrane theory, is not at all clear. In terms of this theory the substances supposedly exist in the cell in a dissolved, diffusable state, and an increase in the permeability of the surface membrane can in no way affect their capacity for interaction. The only event which may take place is diffusion of these substances from the cell, leading to loss of essential substances, which would be utterly incomprehensible.

---

* In all probability, due to reversible changes similar in nature to the initial stage of denaturation (see previous chapter).

In terms of the sorption theory, however, the release of substances following excitation has a different meaning. Substances bound to proteins and incapable of reacting suddenly become dissolved, ionized, and therefore chemically active. The liberation of these or other substances from their bond with proteins may release a chain of metabolic processes necessary for cell activity (Nasonov and Aleksandrov, 1943a, p. 597). Similar ideas were later postulated by Szent Györgyi (1947) in relation to muscle activity and by Koshtoyants (1947, 1951), in relation to other cellular elements.

The Mechanism of Distribution of Mineral Substances between the Cell and Environment

The permeability of cells to nonelectrolytes may be understood by studying a series of dilutions in their intra- and extracellular distribution.
160 Analysis of the curve thus constructed may explain the factors concerned in their distribution. By this method Troshin has shown that the most important controlling factors are: first, their lower solubility in the water of the protoplasm as compared with the water of the surrounding medium, and second, their adsorption on protoplasm micelles. At low concentrations of the substance in the medium, adsorption predominates. This results in a higher intracellular concentration than in the medium. At higher concentrations, further adsorption ceases due to saturation of the adsorbing surfaces, and the substance begins to dissolve in the protoplasm water. According to Henry's law this is characterized by a straight linear distribution (see Figure 72 and others).

Troshin, using a similar method to study penetration of various ions into cells, has shown that the same factors control the distribution between protoplasm and the medium. He reached this conclusion on the basis of his own experimental data and those of other investigators. The following examples are given.

Troshin (1948a) studied the distribution of chlorine ions between yeast cells and the environment. Yeasts grown on beer wort were washed with lactose, and transferred to a lactose solution to which sodium chloride was added at various concentrations. The yeasts were kept in these media until the onset of diffusion equilibrium between the chlorine ions in the cells and the surrounding solution. The intracellular chlorine was estimated by its residue in the medium, and was correlated with the protoplasm water.

The results of these experiments are shown in Figure 95. The abscissa shows the concentration of chlorine in the medium (C_s), and the ordinate shows the concentration in the protoplasm of the yeast cells (C_c). The distribution curve (oa) starts from the zero point of the coordinates. This means that there is no bound chlorine in the yeast protoplasm, and if there is no chlorine in the medium, all the chlorine must also leave the yeast cells. Furthermore, the distribution curve is rectilinear from its very beginning*. This means that chlorine is not adsorbed by the protoplasm

---

* Troshin explains the slight deviation of the curve oa from the rectilinear, in the region of high concentrations, by the fact that after dehydration of protoplasm, the partially bound water is first to leave, as a result of which the amount of substances dissolved in the remaining water is decreased.

micelles and is present there only in the form of a solution, according to the law of Henry. If protoplasm water and the surrounding medium dissolved chlorine to the same extent, then after equilibrium the intra- and extracellular concentrations would be equal (distribution coefficient K = 1)
61 and the distribution curve would correspond to the bisectrix of the angle of the coordinates—ob. In reality this is not so. The distribution curve oa lies beneath the bisectrix at a more acute angle to the abscissa. This means that chlorine is less soluble in protoplasm water than in the medium, and the distribution coefficient K = 0.45.

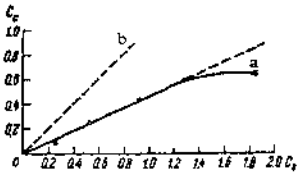


FIGURE 95. Relationship between the concentration of chlorine ions in yeasts (C_c), and that in the medium (C_s) (according to Troshin, 1956)

ob—bisectrix; oa—distribution curve.

Analysis of the distribution curve of chlorine shows it to be very similar to that of acid dyes, which, like chlorine, are almost not adsorbed in the protoplasm, exist in a partially dissolved state and are even less soluble than in the medium (K = 0.25; Figure 80, ob)*.

Fenn, Cobb and Marsh (1934) using the same method, studied the movement of chlorine and sodium between sartorius muscles of the frog and the environment (Figures 96 and 97).

To facilitate comparison with this data, Troshin calculated the ion content, relating it not to 100 g of tissue as done by Fenn and co-workers, but to the water of the muscle fibers. He used the same scale to indicate the ionic concentration of the medium on the abscissa, and that of the cell on the ordinate. Figure 98 shows that chlorine is distributed between the medium and the muscles in exactly the same manner as in the case of yeasts (compare with Figure 95). The distribution curve of chlorine (Figure 98) starts from the point of intersection of the coordinate axes, and continues in a rectilinear fashion with a slope corresponding to a distribution coefficient K = 0.30. This means that all the chlorine in the muscles is dissolved, and if the medium is chlorine-free it diffuses out completely. Consequently it is not bound to protoplasm proteins. Whatever its content in the medium it is always 70% lower in the muscle. As seen in Figure 98 the sodium curve starts somewhat higher than the point of intersection of the axes. Consequently, when the surrounding solution is sodium-deficient, a certain amount remains in the muscle, indicating
162 that a small fraction is firmly bound to protoplasm**. The sodium curve is parallel to the chlorine curve. This means that the solubility of both ions in protoplasm water is approximately the same (K = 0.3).

According to Fenn et al. (Fenn, Cobb, Hegnauer and Marsh, 1934/1935), chlorine and sodium show the same distribution with respect to the sciatic

---

* Note that the chromophore of the acid dye is an organic anion.

** Fenn and co-workers did not pay attention to this fact and assumed that all the sodium in the muscles is free. Subsequently, Troshin (1956, 1957) using labeled atoms, showed that in the muscle fibers of frogs, part of the sodium is not capable of exchange with that in the surrounding solution and is probably firmly bound to protoplasmic proteins.

nerve of the frog (Figures 99 and 100). Here, too, the distribution of both ions is expressed by a straight line starting from the point of intersection of the axes. Consequently, both ions are dissolved in the nerve*. The slope of the curves is such that at any concentration of the chlorine ions in the medium their concentration in the nerve is 57% of the former. The authors assume that all the sodium chloride in the nerve is located in the intercellular spaces. Thus, the magnitude of these spaces, calculated for chlorine, should comprise 57% of the whole nerve. From a purely morphological point of view this figure is obviously exaggerated. The error of
163 this assumption is also evident from the following considerations. In a fresh nerve its "chlorine space" is approximately 30%. After 5 hours in Ringer's solution it increases approximately twofold, while the "sodium space" remains more or less unchanged. Thus, if in the case of muscle fibers a portion of the protoplasm sodium is probably bound to the protein substrate, binding of this kind has not yet been proved for nerve fibers. Nevertheless, there are tissues in which almost all the intracellular sodium is bound. Thus, Abelson and Duryee (1949), using radioactive sodium (Na^{24}), have shown that 88% of sodium in frog eggs is not capable of exchange and is bound to protoplasm, while only 12% exists in the dissolved state and capable of exchange. It is interesting that the action of any harmful agents liberates the bound sodium of the eggs, which may then exchange with the sodium of the surrounding medium.

Steinbach (1940b) investigated the movement of potassium between the cells and the surrounding medium. He immersed sartorius muscles of the frog in Ringer's solution, or in sugar solution containing various concentrations of potassium, and estimated the potassium concentration in the muscles after equilibrium was attained. The results of the experiments are shown in Figure 101**. The curve oab is the potassium distribution curve. In the absence of potassium from the surrounding liquid, a considerable amount remained firmly bound to the protoplasm (oa = 7.5 m equiv percent). With increased concentration of potassium in the medium, the level in the muscles began to increase, corresponding to the section of the curve ab. The shape of this section of the curve indicates that the dependence of C_c on C_s obeys the same law as in the case of nonelectrolytes (sugars, creatinine), aminoacids, and vital stains, namely: $C_c = C_s K + A$. At the beginning the curve has a bent shape—this is the region where adsorption or reversible chemical binding prevails. Later on the curve becomes rectilinear. This indicates that the value of A at these concentrations of potassium in the medium ($C_s > 0.5$ m equiv. percent) becomes constant ($A = A_\infty$), since here the limit of adsorption or chemical saturation of the micellar surfaces is reached. This limit equals the section ae (4.5 m equiv percent). The last rectilinear segment of the curve indicates that the concentration of the potassium dissolved in the protoplasm water increases, according to Henry's law. The line oc parallel to eb illustrates the

---

* Judging from the data given (Figure 99) it may be assumed that a small part of the sodium in the nerve is bound (approximately 1 m equiv. per 100 g of nerve).

** For convenient comparison with other distribution curves of Troshin, the graph plotted from the data of Steinbach was recalculated for the water of muscle fibers.

relationship between the potassium dissolved in the fiber water and its concentration in the medium. The slope of the curve indicates the value of the distribution coefficient (K = 0.45). In other words, the solubility of potassium in protoplasm water is 55% lower than in the medium.

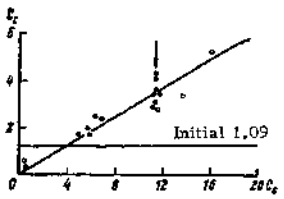


FIGURE 96. Relationship between the concentration of chlorine ions in frog muscles, (C_c, in m equiv per 100 g of tissue), and that in the medium (C_s, in m equiv. percent)(according to Fenn, Cobb and Marsh, 1934/1935)

The arrow indicates the concentration of the substance in the tissue which has been placed in Ringer's solution. Initial-concentration of substance in the tissue immediately after its removal from the organism.

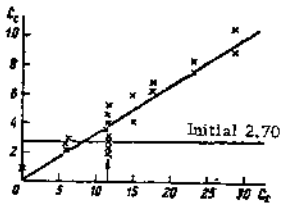


FIGURE 97. Relationship between sodium ion concentration in frog muscles (C_c, in m equiv. per 100 g of tissue), and that in the medium (C_s, in m equiv. percent) (according to Fenn, Cobb and Marsh, 1934/1935).
Legend as in Figure 96.

Thus, analysis of the distribution curve of potassium in muscles shows that potassium exists in muscle fibers in three states. A considerable part of it is firmly bound to the substrate (7.5 m equiv. percent) and may leave the cell only after excitation, damage, or death. This fraction will later be designated by the letter "e" expressing it in m equiv. percent of the protoplasm water. A second part is reversibly adsorbed or bound
164 by an easily reversible chemical bond—A_∞ (4.5 m-equiv. percent). Finally, a small fraction of potassium exists in the form of a free solution (1.13 m equiv. percent at a potassium concentration in the surrounding medium of 2.5 m-equiv. percent). Only this last fraction is in diffusion equilibrium with the external medium. This explains the cases of so-called diffusion of potassium against a concentration gradient. The concept of such a paradoxical phenomenon has been formulated only because the majority of physiologists consider all cellular potassium as freely dissolved in the protoplasm, while in reality a major part of it is bound to the protein substrate.

In another study Steinbach (1937) investigated the distribution of potassium between muscles of the Tenthoidea Thyone briareus, in sea water

containing this element at various concentrations. The results are given in Figure 102*.

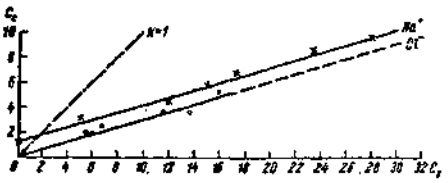


FIGURE 98. Relationship between the sodium and chlorine ion concentrations in muscle fibers (C_c, in m equiv. percent in the water of the muscle fibers), and those in the medium (C_s, in m equiv. percent) (according to Fenn, Cobb and Marsh, 1934; from Troshin, 1956)

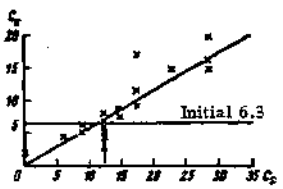


FIGURE 99. Relationship between the sodium ion concentration in the sciatic nerve of the frog (C_c, in m equiv. per 100 g of nerve), and that of the surrounding medium (C_s, in m equiv. percent). Duration of experiment—5 hours (according to Fenn, Cobb, Hegnauer and Marsh, 1934)
Legend as in Figure 96.

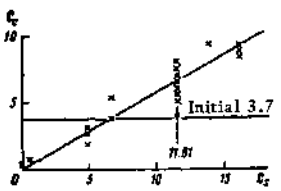


FIGURE 100. Relationship between the chlorine ion concentration in the sciatic nerve of the frog (C_c, in m equiv. per 100 g of nerve) and that in the surrounding medium (C_s, in m equiv. percent). Duration of experiment—5 hours (according to Fenn, Cobb, Hegnauer and Marsh, 1934)
Legend as in Figure 96.

---

* As in other cases, the graph was recalculated by Troshin, taking into account the muscle water, and calculating the intercellular spaces as 17.8%.

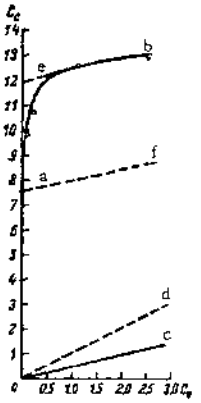


FIGURE 101. Relationship between potassium ion concentration in muscle fibers of the frog (C_c, in m equiv. percent) and that in the surrounding medium at equilibrium (C_s in m equiv. percent) (according to data of Steinbach, 1940b; from Troshin, 1956)
For explanation see text.

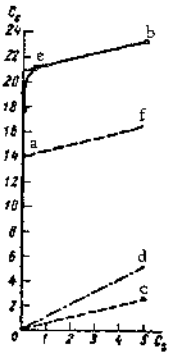


FIGURE 102. Relationship between potassium ion concentration in muscle fibers of holothurians (C_c, in millimoles per 100ml of intracellular water and in sea-water; C_s, in millimoles per 100ml) (according to data of Steinbach, 1937; from Troshin, 1956)
For explanation see text.

The same three forms of potassium in protoplasm may be found here. The potassium which in the complete absence of potassium from the medium does not leave the protoplasm is firmly bound to the substrate (0a); its
165 concentration is 14.0 m equiv. percent. Next, potassium may be reversibly bound to the substance (ad), the concentration of this potassium upon maximum saturation of the surfaces being 6.9 m equiv. percent. Finally, there is the potassium dissolved in the protoplasm (eb). The slope of the curve eb (or oc) indicates that its distribution coefficient between the water of the medium and the water of the protoplasm is 0.48.

Fenn, Cobb, Hegnauer and Marsh (1934) studied the potassium content of frog nerve at various concentrations in the medium. The results of their work are illustrated in Figure 103, which shows a distribution curve of potassium in the nerve of (a) spring and (b) autumn frogs. In both (in a

potassium-free medium) this element remains in the protoplasm in considerable concentrations and is not washed out. This potassium is bound to the substrate. Its concentration in the nerve, as can be seen from the figure, approximately equals 3.0 m equiv percent in both spring and autumn frogs. On increase of the potassium concentration in the medium, the potassium content of the nerve increases too, according to curve a in spring frogs, and curve b in autumn frogs. This potassium is easily exchangeable. Unfortunately, the authors did not have sufficient data relating to low concentrations of potassium in the medium, as a result of which this fraction of potassium cannot be divided into adsorbed (A_{∞}), dissolved, and firmly bound potassium. However, the slopes of curves a and b permit the value of the distribution coefficients of dissolved potassium to be established. In the case of spring frogs it is considerably higher than in autumn frogs (i.e. the curve is steeper). In other words, potassium is more soluble in the protoplasm of the nerve of spring frogs than in that of autumn frogs. Accordingly, the initial level of potassium in nerves of the former (4.8 m-
166 equiv. per 100 g of nerve) is higher than in the latter (3.02 m equiv. percent).

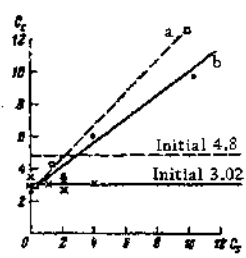


FIGURE 103. Relationship between potassium ion concentration in frog nerve (C_c, m equiv. per 100 g of nerve), and that in the surrounding medium (C_s in m equiv. percent) (according to Fenn, Cobb, Hegnauer and Marsh, 1934)

a—spring frog nerve;
b—autumn frog nerve.

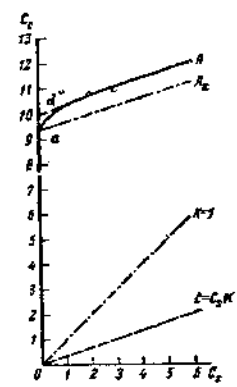


FIGURE 104. Relationship between magnesium ion concentration in frog muscles (C_c, millimoles/liter) in the medium (C_s, millimoles/liter) (according to Fenn and Haege, 1942; from Troshin, 1956) For explanation see text.

The authors found similar distribution curves for potassium in crab and lobster nerves. Here, too, potassium seems to exist in two states in the nerve fibers—in the free and in the bound state.

Figure 104 shows a magnesium distribution curve* between frog muscles and the medium, obtained by Fenn and Haege (1942). The major part of muscle magnesium is bound. With a magnesium-free medium 9.4 millimoles of magnesium were found per one liter of intracellular water, comprising 80-90 % of the total cell magnesium (9.4). Only 0.6 millimoles of reversibly adsorbed magnesium (ad) was found per liter. There is little dissolved magnesium in protoplasm and its distribution coefficient (k) is 0.35.

This concludes the chapter on cellular permeability, which deals with penetration of electrolytes into cells and their distribution between protoplasm and medium. The conclusions form the basis of our theories on bioelectric phenomena.

They are as follows:

1. All electrolytes penetrate cells at more or less equal rates.

2. Intracellular electrolytes exist in three different states:

a) firmly bound to protoplasm, the electrolytes being undissociated and incapable of easy exchange with the surrounding medium; this includes the major part of the cell potassium, magnesium, calcium and phosphates, and possibly a small amount of sodium.

b) adsorbed or bound with labile chemical bonds, easily reversible; this includes part of all cell cations.

c) as free ionized solution in protoplasm water; this includes a small fraction of potassium and phosphates, almost all the intracellular sodium ions and all the chlorine ions.

3) On damage or excitation the nonionized fraction of potassium and phosphates, firmly bound by the protoplasm, is released and passes into the dissolved, ionized state. This transition is reversible. After excitation, or after the reversible damage is arrested, the potassium and phosphates released are again bound by the protoplasm.

4. The movement of nonbound, freely soluble electrolytes between protoplasm and the medium obeys the same laws as nonelectrolytes. During penetration of electrolytes from weak solutions into the cells, adsorption or easily reversible chemical binding prevails in the protoplasm, due to which the distribution graph is at first curvilinear. In more concentrated solutions, adsorption or reversible chemical saturation of the micellar surfaces of the protoplasm is noted. The distribution graph then becomes rectilinear, and on further increase in the external concentration, the slope of the straight line is determined by the distribution coefficient (K) according to Henry's law.

5. Solubility of ions in protoplasm water is lower than in the water of the surrounding medium. The equilibrium established between the concentration of ions in the cell water, and their concentration in the water of
167 the medium, is determined by the distribution coefficient (K), which is always less than one.

6. After damage or excitation, protoplasm loses its phase properties, and the solubility of its ions increases. Due to this, chlorine and sodium ions are directed into the cell. This process is reversible.

---

* The curve was plotted by Troshin from the data of Fenn and Haege, with the magnesium content recalculated per 100 g water of muscle fibers.

7. The direction of diffusion of electrolytes (into the cell, or from it) is determined by increase in the distribution coefficient, either towards the medium or towards the cell.

8. The movement against the concentration gradient, described in the literature, is usually based on the erroneous concept that all cell electrolytes exist in a free, dissolved state.

Chapter 3. Phase Properties of Protoplasm

Immiscibility of Living Protoplasm with Surrounding Water. Attempts to Explain This Phenomenon

If protoplasmic surface membranes are not to be ascribed an insulating function, it must be assumed that living protoplasm is not a simple aqueous solution of proteins and other substances. Furthermore it should then be regarded as a "liquid phase" immiscible with water, and possessing solvent properties differing from it. This would explain why protoplasm, like a drop of oil, does not mix with water, and why all the substances studied dissolve in it differently; the process is then determined by a distribution coefficient less than one, and varies with different substances. In other words, a dilemma exists: either a semipermeable membrane exists at the surface of the cell, or protoplasm is regarded as endowed with phase properties in relation to water.

Until recently, the latter assumption met with difficulties. Indeed, how can protoplasm be a phase in relation to water if it is itself 80% water? These difficulties were overcome in different ways by different investigators. Thus, Lepeshkin (1936, 1937, 1939), suggested the hypothesis of the lipoprotein complex. In his opinion, living protoplasm is a liquid consisting of proteins loosely bound to lipoids, with water dissolved in this complex. This lipoprotein complex, which the author named vitaid, is supposedly easily split by stimulants or noxious agents.

Fischer and Suer's (1935, 1938, 1939) hypothesis was similar, i.e. that protoplasm is a special fluid body, into the composition of which giant protein molecules enter, possessing properties of acids and bases bound to water and mineral substances. The authors supposedly obtained a model of such a compound on mixing sodium caseinate and casein chloride solutions*.

However, in the last decades, discoveries in colloid chemistry made it possible to explain the phase properties of living protoplasm without calling on unproven hypotheses. For example the discovery of the so-called coacervate systems by Bungenberg de Jong and Kruyt (1930); Bungenberg
168 de Jong (1932, 1937a, 1937b). Hitherto it was thought that with decrease in the charge of the particles or upon their denaturation, the liquid hydrophilic colloid system (sol) may either precipitate (coagulation), or solidify in the form of a solid, elastic body (gel). However, a third possibility exists: that the colloid system under the influence of reagents causing desolvatation or removal of the charge remains liquid but divides into two

---

* The colloid systems obtained by them have similar properties to coacervates.

immiscible liquid layers different from each other only in the concentration of the dissolved colloid. Two aqueous colloid solutions arise, separated from each other by a sharp boundary surface; the more concentrated one was called "coacervate", while the other, the more liquid, was called "equilibrium solution". It is interesting that the coacervate may contain a considerable amount of water, exceeding 4 to 5 times the weight of the dry residue.

Simple coacervates consist of one kind of colloid particles, and complex ones are formed by the interaction of several differently charged colloids.

In the opinion of Bungenberg de Jong, and Kruyt, coacervates appear because the colloid particles of the sol approach each other upon decrease of the charge and dehydration, thus the diffusion layer of their hydrate coats is disrupted. Only a layer consisting of strictly orientated particles remains. The orientated aqueous layers coalesce, and all the particles are surrounded by one common hydrate coat. Therefore, all the water of the coacervate is bound, explaining firstly its inability to mix with surrounding "unorganized" water, and secondly the low capacity to dissolve various substances*. Thus, a physico-chemical model was created, reproducing one of the most enigmatic aspects of protoplasm, i.e. its phasic property. In other respects, also, coacervate drops are very similar in their external appearance to protoplasm fragments, or to unicellular organisms devoid of an outer membrane (Figure 105). Under various stimuli, e.g. low temperatures, acids, alkalis, hydrocarbons, alcohols, aldehydes, 169 ketones, ethers and electric current application, vacuoles are formed in coacervate drops, similar to the formation of vacuoles and granules in plant and animal cells. The surface tension of coacervates is approximately the same as in animal and plant protoplasm (Ruiter and Bungenberg de Jong, 1947; Dervichian, 1949). Since the surface tension of coacervate drops differs from that of water, films form on their surface, consisting of orientated colloid particles. However, according to Bungenberg de Jong (1932) and others, these films can in no way affect the diffusion of substances through them, since they consist of sparsely distributed and well-hydrated particles.

All these facts make it highly probable that in terms of colloid structure, living protoplasm is a complex coacervate. This postulate is acceptable to an ever-increasing number of investigators (Bungenberg de Jong, 1932; Duclaux, 1934, Guilliermond, 1941; Oparin, 1941; Nasonov and Aleksandrov, 1934 a; Makarov, 1948 a, 1950; Danzhar, 1950; Troshin, 1953, 1956, and others).

Distribution of Substances between Coacervates and Their Equilibrium Liquid

Experiments on the distribution of substances between coacervates and the surrounding liquid are especially important in permeability studies, but they are still scarce. Holleman, Bungenberg de Jong and Modderman (1934) studied the distribution of alcohol, resorcin, sodium sulphate and

---

* The concept of coacervate formation formulated by Bungenberg de Jong and Kruyt has been criticized by Mikhailov (1935), Lepeshkin (1939), Dervichian (1949) and others.

potassium iodide between a simple gelatin coacervate and its equilibrium liquid. Their data are compiled in Table 27, the concentrations of the substances being recalculated per 100 g of water. The distribution coefficients (Q) between the coacervate and the equilibrium liquid are given.

From the data in Table 27, comparisons may be made between certain features of the nature of distribution of substances (in the case of coacervates) and living cells. Firstly, both cases illustrate a distribution, characterized by different coefficients (Q) for different substances, rather than a simple equilibration of concentrations. For sodium sulphate, Q is less than one, while for resorcin it is greater than one. Secondly, the value of Q changes with the concentration of the substance. With increased concentration of sodium sulphate and resorcin, the value of Q decreases. This indicates not only solubility in the water of the coacervate, but also adsorption to its micellar surfaces. The authors themselves reached the same conclusion. Unfortunately, they do not give a sufficient number of points (concentrations) for the construction of a distribution curve.

The studies of Troshin (1951a) on the distribution of substances between the coacervate and the medium are of special interest, since they were performed by exactly the same method as in his work with living organisms. In his studies he employed a complex coacervate prepared from gelatin and gum arabic, to which a certain amount of hydrochloric acid was added. The distribution of galactose and saccharose was studied. These substances were chosen because, according to available data, they do not
170 affect the coacervates. In addition, their distribution was well studied by Troshin on living cells (yeasts, muscles). As in the studies on complex living organisms, the time of onset of diffusion equilibrium was first determined. In the case of sugars, diffusion equilibrium occurred earlier than 15 hours. On this basis Troshin's experiments lasted 17-19 hours. Subsequent analysis of the sugar was performed and the amount of water and dry residue in the coacervate were determined. Galactose and saccharose, at the concentrations employed, did not substantially affect the hydration of coacervates.

This observation agrees with data of other investigators. With the data obtained, the concentration of sugar was determined in the water of the equilibrium liquid and of the coacervate. Distribution graphs were plotted (Figures 106 and 107). These show a striking resemblance to sugar distribution graphs between live protoplasm and the environment (erythrocytes—Figure 72, muscles—Figure 74, yeasts—Figure 80, etc.), (creatinine—Figure 78, alanine—Figure 86, vital stains—Figure 90). Analysis of these curves shows that the same factors control the distribution of substances between coacervates and the medium as control the distribu-
171 tion between living organisms and the medium. Indeed, in its initial phase, corresponding to low concentrations, the distribution curve rises steeply and is located above the bisectrix oa, corresponding to concentration equilibrium. This means that in this region of the curve the substance penetrates the coacervate at a higher concentration than that in the surrounding medium at equilibrium. It is obvious that in this region, adsorption to the coacervate micelles predominates. Later, the curve crosses the bisectrix oa and continues in rectilinear form at an angle less than 45° to the abscissa. In Troshin's opinion the process of adsorption stops here due to saturation of the surfaces, and subsequently only solution of the substance in the coacervate water occurs.

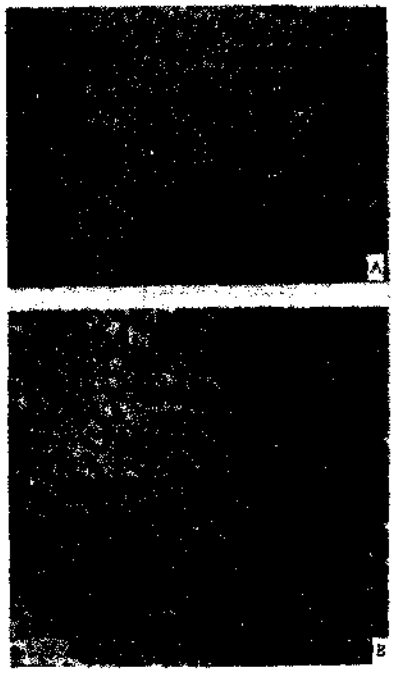


FIGURE 105. Vacuolation of coacervate drops prepared from gelatin and gum arabic, after cooling (according to Troshin, 1948b)

A—coacervate drops at 40° C; isolated vacuoles are seen; magnified 25 times, in transmitted light; B—coacervate drops at 20° C, the drops are markedly vacuolated. X 300, with lateral illumination.

Troshin showed that in case of coacervates the distribution curve, as in the case of living tissues, corresponds to the equation

$$C_c = C_s K(1 + \frac{A_\infty}{C_s K + a}),$$

where C_c is the concentration of the sugar in the coacervate, C_s is its concentration in the medium, and K is the distribution coefficient according to Henry's law, which equals the ratio of concentration in the coacervate water to that in the water of the medium. A_∞ is the limit of saturation; a is a constant determining the slope of the curve. In galactose distribution in the coacervate, K equals 0.61. For saccharose K equals 0.60. This means that in the coacervate water, sugars dissolve by 40% less than in the surrounding medium. This is understandable if one assumes that the water of coacervates is totally bound, the molecules being orientated by the coacervate micelles.

A_∞ (limit of adsorption) numerically equals the sector od on the ordinate axis. In our case this equals 0.22 g per 100 ml of coacervate water, or per 18 g of its dry residue in the case of both sugars.

As on analysis of the distribution curve in protoplasm, the distribution of sugars between coacervates and medium may be divided into two components. The straight line oc illustrates the distribution of the dissolved fraction of the sugar, according to the equation $x = C_s K$. The adsorbed part of the sugar is found by subtraction of the dissolved sugar from the total sugar of the coacervate (C_c). The adsorbed sugar (A) will then be expressed by the equation

$$A = C_c - C_s K.$$

Figure 108 shows a curve obtained in this manner. This curve strikingly resembles the adsorption curves of sugars obtained by Troshin for erythrocytes (Figure 73) and for yeasts (Figure 81).

The data on distribution of sugars between coacervates and the medium given, and comparison of the resultant curves with similar curves for living objects, again confirm the hypothesis that protoplasm is a complex

Table 27

Distribution of sodium sulfate and resorcin between the coacervate and equilibrium liquid (according to Holleman, Bungenberg de Jong and Modderman 1934; from Troshin, 1956)

Sodium sulfate			Resorption		
% in water of the equilibrium liquid (C_c)	% in water of the coacervate	$Q = \frac{C_c}{C_s}$	% in water of the equilibrium liquid (C_c)	% in water of the coacervate	$Q = \frac{C_c}{C_s}$
10.8	9.1	0.83	4.2	10.7	2.55
14.0	8.9	0.64	5.9	14.5	2.50
17.4	10.7	0.61	17.5	33.7	1.90
21.2	11.1	0.52	30.4	40.1	1.33

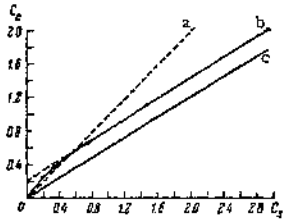


FIGURE 106. Relationship between concentration of galactose in the coacervate (C_c, in g per 100 ml of coacervate water) and that in the equilibrium liquid (C_s, percent) (according to Troshin, 1951a) Legend as in Figure 72.

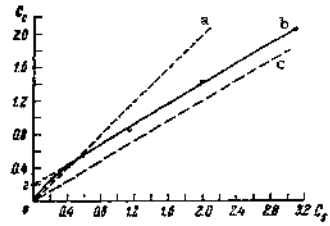


FIGURE 107. Relationship between concentration of saccharose in the coacervate (C_c, in g per 100 ml of coacervate water) and that in the equilibrium liquid (C_s, percent) (according to Troshin, 1951a) Legend as in Figure 72.

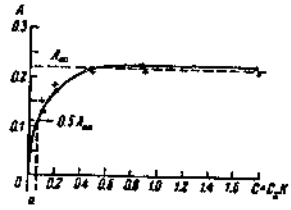


FIGURE 108. Adsorption isotherms of galactose by coacervate colloids (according to Troshin, 1956)

On the abscissa–concentrations of galactose dissolved in the coacervate water (in %); on the ordinate– amount of adsorbed galactose in the coacervate (in g). Experimental data designated by circles; data calculated from Langmuir's formula designated by crosses.

172 coacervate in its physical-chemical structure, and that distribution of substances between cells and the medium is determined mainly by this structure.

Chapter 4. Discussion and Conclusions

The study of data on cellular permeability forced us to refute the theory based on the concept of semipermeable membranes on cell surfaces. Among all the arguments against the membrane theory of permeability we consider the following to be the most convincing.

1. On immersion of the cell in solutions of different concentrations, the cells change their volume and do not obey the law of osmosis. Deviations from this law in the direction of insufficient loss of water are usually explained by the presence of osmotically inactive, bound water.

Deviation from the law of osmosis, observed by us in the direction of an excessive loss of water in macromolecular solutions, cannot be explained by a membrane mechanism. These facts alone cast doubt on the existence of semipermeable membranes. Even if the theory of selective diffusion through membranes is not accepted, but is replaced by the theory of selective "pumping off" of substances from the cell, the cells should obey the law of osmosis, which in reality does not happen.

2. An absolute requirement of the membrane theory is that all intercellular substances, especially minerals, be present in living protoplasm in a dissolved state, capable of diffusion. The sum of osmotic pressures of all these substances should equal the osmotic pressure of the surrounding aqueous solution. Data recently obtained indicate that this assumption is incorrect. The study of proteins isolated from cells, and especially experiments on the specific radioactivity of labeled ions penetrating the cell, show that only a small fraction of protoplasm electrolytes exists in the free state, while the remaining electrolytes are bound to the protein substrate, are not ionized and are not diffusible. Numerous data indicate that the major part of cell potassium, phosphorus, magnesium and calcium, and possibly a small part of sodium, are bound in this way. Only cell chlorine exists wholly in the free state.

3. The membrane theory states that certain substances cannot diffuse across the cell membrane, while others diffuse only very slowly. The explanation of the dissimilar electrolytic composition of protoplasm and the surrounding medium (nonequilibrium of solutions in the cell and in the medium) is based on this assumption. Among substances which do not penetrate the cell membrane, the membrane theory also includes such vitally important compounds as sugars, aminoacids, and many electrolytes (Na^+, Ca^{++}, Mg^{++}, Cl^-, HPO_4^- and others).

This postulate of the membrane theory was also disproved by recent data obtained by direct chemical analysis of tissues and of suspensions of cell elements (yeasts, erythrocytes), as well as by analysis of the contents of isolated muscle and giant nerve fibers, and by the method of labeled atoms. By these methods it has been established that all the substances

173 studied, including sugars, aminoacids and electrolytes, penetrate the cell sufficiently rapidly and approximately at the same rate, but the equilibrium concentration levels inside protoplasm differ widely, depending on the nature of the substance and its concentration in the medium.

4. Passage of certain substances from the cell after excitation (potassium, phosphates) is explained, according to the membrane theory, by increase in permeability of the membranes ("loosening"). However, the reentry of these substances into the cell after cessation of the stimulus could not be explained by the membrane theory, since decrease in permeability of the membrane when the cells return to a resting state may only render less possible the return to the protoplasm of substances which have left it.

All these data which do not fit into the framework of the classical membrane theory caused many of its proponents to introduce modifications. One of the most important attempts of this kind was the rejection of the concept of passive semipermeability of the membrane based on the "sieve" principle, or selective solubility of substances in the membrane (lipid solubility), and its substitution by hypotheses on selective pumping off mechanisms. According to the latter, substances travel to and from the cells to the surrounding medium by means of metabolic processes. The theory of active transport of sugar molecules across the membrane by special enzymes and carriers localized in the cell membrane, which has been discussed above, belongs in this category (Rozenberg, Wilbrandt and others).

The theory of Lundegard and Krogh should also be included here. According to this the membrane consists of rodlike molecules, distributed in the form of a palisade the tips of which are capable of attaching sodium or potassium ions, and transporting them either into the cell, or from the cell into the surrounding medium, as the necessity arises, by rotating 180° Finally, the theory of the "sodium pump" of Hodgkin et al. comes into this category.

All these theories are highly speculative and are not even supported by model analogues. In this respect they differ from the classical membrane theory which, in its original form, took into account all details and was accompanied by a number of excellent physico-chemical models reproducing selective permeability of membranes to different substances. Twenty to thirty years ago the membrane theory could satisfactorily explain all the then known facts. Now, however, this theory is invalid in the face of all the data obtained by modern methods.

The sorption theory of permeability suggested by us is based on a highly probable assumption that living protoplasm is a system of complex coacervates. This explains the fact that protoplasm behaves as a phase, immiscible with the surrounding water. As we have seen, Troshin studied distribution curves of substances between coacervates and the equilibrium liquid and he found that they are similar to the distribution curves of substances between living protoplasm and the surrounding medium. Analysis of these curves made it possible for him to establish a number of factors determining this distribution. It was found that the time necessary to attain diffusion equilibrium between protoplasm and medium is relatively short, and more or less the same for different substances (see Figures 65/67, 69/71, 75, 77, 79, 84, 88, 89, 94). Therefore the definition of
174 "permeability" as the rate of penetration of substances into cells is not specific for different substances. It is therefore of no great value and must

be rejected. The nature of distribution of substances, after equilibrium has been established, is important and scientifically interesting. In this respect the term "cellular permeability" is not convincing, especially in respect to cell membranes, since it implies the necessity of overcoming an obstacle at the cell surface. It would be more correct to allude to the movement of substances between the cells and the medium, but the term "permeability" has been established by usage, and with modifications, may still be used.

The analysis of distribution curves of nonelectrolytes, and of weak and strong electrolytes, made possible the quantitative differentiation of three states in which the same substance may exist in protoplasm: a state in which the substance is firmly bound to the protein substrate; a state of the substance reversibly adsorbed to micellar surfaces; and a state of the substance freely dissolved in protoplasmic water. The amount of the firmly bound fraction is small, depending on its concentration in the surrounding solution. Evidently, substances are chemically hypoactive in this state but can move to a free solution after damage or stimulation. The adsorbed part of the substance is in equilibrium with the dissolved substance. As we have seen, a relatively greater amount is adsorbed from weak solutions. With saturation of the surfaces, adsorption becomes weaker and finally ceases, followed only by increased concentration of the dissolved substance. The intensity of the adsorption of the substance may be described by the adsorption limit (A_∞). Its value may vary for different substances (see Table 28), from zero (e.g. chlorine) to very high values, the attainment of which may be accompanied by poisoning and death of the protoplasm (e.g., with basic dyes, narcotics, etc). Table 28 shows that with the same tissue (sartorius muscles of frog) potassium has the highest adsorbing capacity ($A_\infty = 4.5$), followed by sodium, the adsorbing capacity of which is approximately 4 times less ($A_\infty = 1.2$); then comes magnesium ($A_\infty = 1.0$), followed by the dye phenol red which is approximately 1,000 times less adsorbed than potassium ($A_\infty = 0.003$) and finally, chlorine with an adsorbing capacity of zero. In the calf muscles of the frog, the constants A_∞ may be arranged in the following order of magnitude: saccharose (0.03) < creatinine (0.07) < galactose (0.11) < alanin (0.33) < arabinose (0.6). Thus values within this series vary tenfold.

The value of the constant A_∞ is of the greatest interest to us since in the adsorbed state, when surface catalysis is possible, the substances in protoplasm show the greatest activity; this has been demonstrated by comparing the adsorption curve of sugar in the protoplasm of yeasts, with its decomposition. In the previous chapter it was stated that the sorption level of protoplasm strongly changes in the condition of paranecrosis. Evidently, living protoplasm is capable, with the participation of metabolism, of increasing or decreasing its adsorption level within a wide range, and thus to regulate not only the intensity but also the direction of metabolism. For example, according to data from the biochemists associated with Oparin (Kursanov, 1940; Oparin, 1948; Sisakyan, 1951 and others), certain en-
175 zymes are hydrolytically active in the dissolved state, while in the bound state they lose this ability, thus securing synthesis.

The fraction of the substance dissolved in protoplasm water probably plays a smaller role in metabolism. This fraction is directly dependent on the content in the medium, and is characterized by a distribution coefficient

not connected with the concentration of the surrounding solution. The value K is always less than zero and, as seen from Table 28, does not vary very strongly—from 0.25 to 0.55 (maximum twofold). Even with the same tissue (sartorius muscles of frog) the value of K is not the same for different substances (Table 28). For potassium it is 0.45, for chlorine 0.30 and for phenol red 0.25. It is obvious that the lower solubility of substances in protoplasm, as compared with water, cannot be explained solely by a certain percentage of bound water. If this were so, then in the same tissue K would be the same for all substances. Among the ions studied, potassium showed the highest solubility (0.45), followed by magnesium (0.35), sodium (0.30) and chlorine (0.30). Thus, the constant K varies from substance to substance much less than the constant A_{50}. The latter is most important in understanding the permeability of protoplasm, and as the most significant and interesting from the biochemical point of view.

176 On excitation a part of the firmly bound substances passes into a free solution. Since the latter is in equilibrium with the adsorbed fraction, the amount of the substance adsorbed to micellar surfaces immediately increases. In turn, this should lead to the increase of the corresponding metabolic link. In our opinion, the mechanism by which a chain of biochemical processes performing the work of the cell is initiated in protoplasm is the release of substances bound to protein, after excitation.

Experiments were cited (supra) to show that the release of substances bound to protoplasm proteins occurs after denaturation of these proteins by various agents. These experiment are in accordance with our denaturation theory of stimulation and damage. After excitation protoplasm begins to lose its phase properties, due to which its capacity for dissolving substances increases (K approaches zero). This may explain the diffusion of Na^+ and Cl^- into the cells in the condition of excitation, reversible damage and death.

These are the basic assumptions of the sorption theory of permeability. In conclusion this hypothesis will be compared with that group of theoretical considerations collectively termed the "theory of selectively pumping off mechanisms" (Rozenberg, Wilbrandt, Lundegard, Krogh, Hodgkin and others) and which, for the sake of brevity, will be called "the pump theory"

Both the sorption theory and the pump theory were postulated when the classical theory of semipermeable cell membranes was shown to be inadequate to explain a variety of data obtained by new methods of study. The sorption theory considers the mechanisms regulating the distribution of substances between the cell and its environment to be distributed throughout the protoplasm, and not necessarily connected with its surface. The sorption theory does not, therefore, employ the hypothesis of cellular surface membranes. Such membranes as other border formations (cuticles, bristle borders, membranes of egg cells, etc.) may exist, but they perform certain particular cellular functions and do not play a universal role in movement of substances between cell and medium, which is usually ascribed to cell membranes.

The proponents of the pump theory, however, remain faithful to the idea of the surface membrane, and they localize the hypothetical "pump" within the membrane. It seems to us that this is the weak spot of the theory, since the suggested surface film is usually considered to be very thin (several molecular layers) while the mechanisms of the "pump" should be specific for each of a great number of substances penetrating the cell. It is very difficult to conceive all these mechanisms being localized in such a small space.

Table 28

The value of the distribution coefficient, from Henry's equation (K), of the adsorbed substance (A_∞) and firmly bound substance (D). (A_∞) and D are expressed in millimoles per 100 ml of cell water (according to Troshin, 1956)

Objects	Substance	K	A	D
Coacervates	Galactose	0.61	1.22	—
"	Saccharose	0.60	0.64	—
Yeasts	Lactose	0.55	6.43	—
"	Chlorine ions	0.45	0.00	—
Rabbit erythrocytes	Galactose	0.42	0.16	—
Muscles of frog calf	Arabinose	0.43	0.60	—
The same	Galactose	0.32	0.11	—
" "	Saccharose	0.29	0.03	—
" "	Alanine	0.40	0.33	—
" "	Creatinine	0.38	0.07	—
Sartorius muscles of the frog	Phenol red	0.25	0.003	—
The same	Potassium ions*	0.45	4.5	7.5
" "	Sodium ions**	0.30	1.20	—
" "	Chlorine ions**	0.30	0.000	—
" "	Magnesium ions***	0.35	0.060	0.94
Retractors of galacturia	Potassium ions*	0.48	6.90	14.0

Calculated from the data of:
* Steinbach, 1937, 1940a;
** Fenn, Cobb and Marsh, 1934;
*** Fenn and Haege, 1942.

177 Finally, from the point of view of the "cell pump" theory, continuous pumping of the substance to or from the cell requires constant expenditure of metabolic energy. This is proved by the fact that poisoning of cells by metabolic inhibitors decreases the difference in the concentration of substances within and without the cells. The work of Hodgkin and Keynes (1955) is an example of this kind. They studied the effect of dinitrophenol, cyanides, and cold, on the potassium and sodium content of giant nerve fibers of cephalopod mollusks.

However, experiments of this kind hardly confirm pumping off mechanisms in the cell. The point is that if certain electrolytes leave a damaged or excited cell, and subsequently return in its resting condition, this movement requires energy expenditure, no matter what its mechanism is. The source of the energy is cellular metabolism. It is also known that in the resting condition so-called basic metabolism takes place in the cell. The purpose of this basic metabolism is to maintain the extremely labile structure of protoplasm on which all the properties of the latter depend. Any

disturbance of basic metabolism should lead to changes in the sorption properties of the protoplasm, its capacity for dissolving substances, etc. (Nasonov and Aleksandrov, 1940). . Consequently, in terms of the sorption theory any disturbance of metabolism should cause redistribution of substances.

Thus, the sorption theory, too, maintains that the distribution of substances between the living cell and the medium may occur and be maintained only at the expense of energy supplied by metabolic processes. In this respect there are no differences between the requirements of the two theories. The difference consists only in the meaning—what is the nature of these forces which actually bring about the distribution and redistribution of substances? The pump theory supplies no definite answer to this question. The sorption theory gives a specific answer in respect to the factors of distribution. These are the following: solubility of the substance in the protoplasm water; adsorption to micellar surfaces of protoplasm colloids; and chemical binding to the substrate. All these factors vary and depend to a great extent on the physiological condition of the protoplasm. This determines the level, and nature of distribution of substances between the cell and its environment.

The above refers mainly to cells which are surrounded on all sides by tissue fluid, for example, muscles, nerves, connective tissue cells, etc.

A somewhat different picture is observed with cells of certain glands or epithelia capable of reabsorption. These are cells of border tissues, e.g. the mucous membrane of the alimentary tract, kidney tubules, etc. The special purpose of cells of this kind in the organism is selective pumping of solutions of certain substances in the basal or apical directions, sometimes against the concentration gradient. Morphologically such cells are characterized by well-defined polarity and nonequilibrium of structure. Their nonequilibrium is first of all determined by uneven distribution of chondriosomes, these being most prevalent in the basal part of the cells, 178 and also by the polar location of the Golgi apparatus. It is quite obvious that secretory or resorbing cells should possess some pumping mechanism, working together with the various metabolic processes. However, as already mentioned, it remains completely unexplained why it is necessary to "place" this mechanism within the membrane, as postulated by Rozenberg, Wilbrandt, Lungdegard, Krogh, Hodgkin and others.

It seems to us much more logical to localize the suggested pumping mechanism throughout the whole epithelial cell.

We shall start from those general assumptions which were taken as a basis for the sorption theory. We assume that the protoplasm of secretory cells is, as in other cells, a coacervate system, in the water of which many substances are considerably less soluble than in water of the surrounding medium. Free diffusion across a layer of such cells will be slowed down considerably, and will be possible only in the direction of the gradient. Now let us imagine that molecules, freely diffusing within the cells, and capable of chemically binding and releasing the pumped substance, enter into the composition of protoplasm as a complex coacervate (acceptor). Specific examples are the epithelial cells of kidney tubuli, which resorb glucose, sodium and possibly certain other substances from the lumina of the tubules. In the basal parts of these cells there is a great number of mitochondria distributed in the form of a palisade, and containing various

enzymes. It may be visualized that in the apical half of the cells devoid of mitochondria, reactions binding glucose to acceptor molecules prevail, while in the basal half, with an abundance of mitochrondria, splitting of this complex predominates. Then in the presence of free diffusion of acceptor molecules within the cell, a higher concentration of freely dissolved glucose should be maintained in the basal part of the cell (mitochrondrial part) as compared with the apical part. This concentration may be higher than the equilibrium concentration of glucose in the tissue fluid surrounding the bases of the epithelial cell, as a result of which diffusion of the substance from the cell will begin through its basal surface to the outside.

It is self-evident that such a mechanism is possible only with the participation of intracellular metabolism and energy expenditure.

We realize that all these assumptions may be considered as one of many possible hypotheses, but the advantage of our hypothesis as opposed to others is that we localize the "cell pump" within the whole cell, and not in the semipermeable membrane which is thought to consist of a few molecular layers.

Part III

BIOELECTRIC POTENTIALS

Chapter 1. Phase Theory of Bioelectric Potentials

History of the Problem

The theory of excitation is closely linked to the theory of bioelectric potentials. It is known that the surface of the excited part of a cell is electronegative in relation to the nonexcited part. This gave physiologists the opportunity to study quantitatively, and with great precision, the degree of excitation of the tissue. These studies, using electrical methods, were carried out in the middle of the last century. There are no other methods for study of excitation as accurate as these. This is why the majority of studies on excitation are electrophysiological, and are performed mainly on conducting tissues. In addition, the mere fact of generation of electrical energy by excited protoplasm should illuminate the physicochemical nature of excitation.

The 19th century investigators could not postulate any satisfactory theory of bioelectric potentials. Possibly in those times physicists were unaware of liquid chains which, in the absence of metallic electrodes, could create potential differences, similar in magnitude to biological ones. At the end of the 19th century, papers appeared on the subject of the theory of membrane potentials (Ostwald, 1890; Michaelis, 1925 and others) and phase potentials (Nernst, 1892). These made possible the use of physicochemical methods to explain bioelectrical phenomena. The then predominant concept of protoplasm as an aqueous solution of proteins and salts surrounded by a membrane influenced the physiologists' attempts to construct a general theory of biopotentials. The papers by Bernstein (1912), Höber (1905, 1907, 1926) and others contributed to the formation of, and laid a substantial foundation for, the membrane theory of bioelectric potentials, which, with certain changes and additions, is still the basis of our theories of electrical phenomena in cells.

The phase potentials of Nernst were used by physiologists in these theoretical considerations only for certain small variations in the theory of the membrane structure. One example is the theory of Beutner (1920, 1933), who tried to explain bioelectric phenomena by assuming that the cell membrane consists of two layers of substances insoluble in water: one
180 giving an acid reaction and the other a basic one*.

---

* This theory met with no success, since it was based on a false concept of the similarity of the salt composition of the cell with that of the surrounding medium.

The previous section, discussing permeability phenomena, concluded with a complete denial of the presence of hypothetic surface membranes which, according to the majority of contemporary physiologists, act as semipermeable membranes controlling the uptake of soluble substances by the cell. We started from the concept of the protoplasm as a coacervate system whose water behaves as a phase in relation to the surrounding water. From this we concluded that the distribution of substances between the cells and the medium is not determined by the permeability of the hypothetic membranes, but by the solubility of the substances in the protoplasm, their adsorbance on the protoplasm micelles and by their chemical binding to the protein substrate. We cited data showing that a considerable part of the protoplasm electrolytes is bound to its proteins, and that only a small part is in the free dissolved state and that upon damage or in the condition of stimulation the protoplasm loses its phase properties, releasing the electrolytes bound to it, which pass into a simple aqueous solution. The membrane theory of bioelectric potentials was abandoned by us, since it was based on faulty premises. In its place, the phase theory, based on the concept of protoplasm as a "phase" in relation to the surrounding water, was accepted (Nasonov and Aleksandrov, 1944; Troshin, 1956). Data will now be adduced to support this theory.

Any theory of bioelectric potentials should in the first place explain three basic groups of phenomena. These are: firstly, the appearance of a difference in potential due to the application of salt solutions to the surface of the cell ("salt potentials"); secondly, the appearance of difference in potential between the surface of the damaged and intact parts of the cell ("injury potentials", or "potentials of rest"); thirdly, the appearance of a difference in potential between the surfaces of the excited and resting parts of the cell ("potentials of excitation", or "action currents").

Salt Potentials

The explanation of salt potentials will form the basis of our theory of bioelectric phenomena.

Salt potentials were first described by Höber (1905). The basis of this phenomenon is that on contact of any part of the cell surface with a salt solution, a difference in potential may form at once on contact of this segment with its neighbor (Figure 109 C). If isomolar salt solutions are used, differing by the cation only (for example, chlorides of various metals) or by the anion only (for example, sodium salts of various acids) an ionic series may be obtained by arranging them according to their ability to bring about a state of negativity or positivity in the protoplasm, as compared with NaCl solution.

Höber obtained such series for cations and anions:

$$-K > Rb > NH_4 > Cs > Na > Li +$$

$$\text{tartrate} > SO_4 > HPO_4 > \text{acetate} > Cl > Br > I > NO_3 > CNS +$$

181 He tried to explain them by the relative toxicity of the ions. He assumed that they poisoned protoplasm, with resultant loosening of the membrane and development of negativity. However, on this basis NaCl would be more "toxic" than LiCl, NaBr, NaI and NaCNS, which is of course incorrect.

In addition, it would also have to be assumed that Na penetrated cells more readily than Li, and Cl more than Br and I. This would also contradict the basic assumptions of the membrane theories of biocurrents, according to which Cl and Na do not penetrate at all into cells.

In order to explain salt potentials, the theory of phase potentials of Nernst (1892) and his famous experiments (which are schematically illustrated in Figure 109 A) are taken as a basic premise. A liquid immiscible with water, which we shall further call the phase, was poured on the bottom of a U-tube. Water, which is of a lesser specific weight, was poured onto the surface of this phase, in the right and the left arms of the tube. If electrolytes were dissolved in the water, then at the interphase a difference in potential was detected. Such potential changes occurred in both the right and the left arms of the tube.

This difference in potential was caused, according to Nernst, by the unequal solubility of electrolyte ions in the nonaqueous phase. If the solubility of the cations in the phase is larger than that of the anions, the cations will tend to pass into it in a larger quantity than their anion partners. Of course, a prevalence of cations over anions in the solutions, which can be analytically detected, does not occur, but the tendency to pass into the phase at a higher concentration will be expressed by an experimentally detectable difference in potential, with a negative electrical charge on the side of the aqueous solution.

182 Starting from these considerations, Nernst theoretically developed a formula determining the magnitude of electromotive force formed:

$$E = \frac{RT}{Fm_1} \ln \frac{K^+y^+}{C^+} = -\frac{RT}{Fm_2} \ln \frac{K^- \; y^-}{C^-}$$

where m_1 and m_2 are the valencies of the ions; K^+ and K^- are the distribution coefficients of the cations and anions between the two phases; C^+ and C^- are the concentrations of cations and anions in the aqueous phase; y^+ and y^- are the concentrations of the cations and anions in the nonaqueous phase.

Thus, for electrolytes with ions of the same valency, there is the formula:

$$E = \frac{RT}{Fm} \ln \frac{K^+y^+}{C^+};$$

$$E = \frac{RT}{Fm} \ln \frac{K^-y^-}{C^-}.$$

Combining the right hand and left hand parts of these equations results in:

$$E = \frac{RT}{2Fm} \ln \frac{K^+y^+C^-}{K^-y^-C^+},$$

and since in solutions, C^+ should be equal to C^- and $y^+ = y^-$, then

$$E = \frac{RT}{2Fm} \ln \frac{K^+}{K^-};$$

for monovalent electrolytes

$$E = \frac{RT}{2F} \ln \frac{K^+}{K^-}. \qquad (1)$$

If now two different electrolyte solutions with a common anion and different cations are placed on two different sectors of the phase (Figure 109 A), the difference in potential in these two points (right and left arms of the tube) will be determined by the following formula:

$$E = E_1 - E_2 = \frac{RT}{2F}\left(\ln \frac{K_1^+}{K_1^-} - \ln \frac{K_2^+}{K_1^-}\right) = \frac{RT}{2F} \ln \frac{K_1^+}{K_2^+} \qquad (2)$$

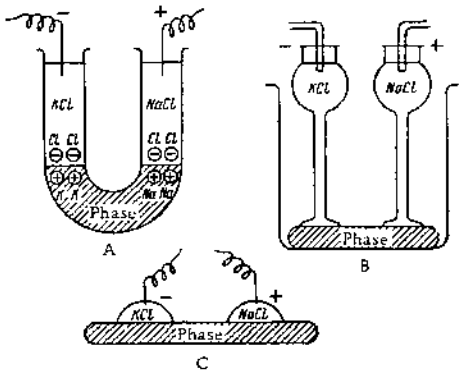


FIGURE 109. Salt (phase) potentials in the chain: aqueous solution of KCl—phase—isomolar aqueous solution of NaCl

Phases: A—a liquid immiscible with water; B—coacervate; C—protoplasm.

In other words, the magnitude of this potential difference is proportional to the logarithm of the ratio between distribution coefficients of the cations between water and the phase. Consequently, in the presence of the same anions the cations should give a series according to their degree of solubility in the phase. The more soluble ones will convey greater negativity to the surface.

As shown by experiment, different phases (for example, phenol, guaiacol, cresol, and others) give different series (Beutner, 1920). In our opinion, protoplasm is a coacervate system. Consequently, the role of the phase should be played by the solvate water of the coacervate. If this assumption is correct, we should obtain salt potentials at the surface of the coacervate arranged (in the presence of different cations) according to the degree of negativity, in series similar to the series of salt potentials on the surface of living protoplasm described by Höber.

183 In order to check this assumption, Troshin (1948b) studied salt potentials on complex coacervates, which, according to Bungenberg de Jong (1932), Oparin (1941) and others, most closely resemble protoplasm. Such a

coacervate was prepared by Troshin from gelatin and gum arabic, and a liquid was obtained immiscible with the equilibrium solution water, delineated from it by a sharp boundary. The coacervate was placed on the bottom of the vessel, and its surface brought into contact at two points, with an aqueous $\frac{N}{10}$ solution of two chlorides. The vessels into which the salt solutions were poured were connected by agar bridges with calomel electrodes (Figure 109B). The difference in potential, formed at the points of contact of the coacervate with the salt solutions, was determined by a mirror galvanometer, sodium chloride being always present in one of the vessels. Chlorides of various monovalent cations, in $\frac{N}{10}$ concentrations, gave a certain series of values of the potentials obtained (Table 29).

For comparison with the series obtained, $\frac{N}{10}$ solutions of the same chlorides were placed at two points on the surface of calf muscles of the frog, and the differences in potential were transmitted to the mirror galvanometer by calomel electrodes (Figure 109C).

Table 29

Differences in potential of $\frac{N}{10}$ salt solutions obtained on contact with the coacervate, and with the living muscle, in relation to an $\frac{N}{10}$ solution of NaCl (according to Troshin, 1948b)

$\frac{N}{10}$ solution	Differences in potentials (millivolts)	
	on the coacervates	on living muscle
KCl	− 18.7	− 26.7
RbCl	− 16.6	− 16.5
NH_4Cl	− 15.8	− 13.3
CsCl	− 14.9	− 11.2
NaCl	0.0	0.0
LiCl	+ 10.7	+ 6.5

It follows from Table 29 that cations of the chlorides formed at the surface of the complex, coacervate exactly the same series of negativity as on the surface of living muscles. Attention should be paid not only to the coincidence of the order of arrangement of the cations −KCl > RbCl > NH_4Cl > CsCl > NaCl > LiCl+, but also to the closeness of the absolute values of the potentials (only the potential of KCl on muscles is considerably higher than on the coacervate). This coincidence is the more remarkable since on contact with the equilibrium liquid of the coacervate, differences in potential were also formed, but they were of a considerably smaller absolute magnitude, giving another series in respect to the negativity: −CsCl > RbCl > KCl > NH_4Cl > NaCl > LiCl+, where the cations are arranged

according to mobility. In other words, a typical series of diffusion potentials was obtained. The same series was obtained with killed muscle.

All this confirms the hypothesis that the differences in potential arising from contact of salt solutions with living protoplasm are nothing but the phase potentials of Nernst, the role of the phase in relation to water being played by the coacervate, i.e. protoplasm*.

Thus, the main causes of salt currents are the jumps in potential at the boundary between the phases, arising as a result of unequal solubility
184 of the cation and anion in protoplasm. To this potential may be added another, the diffusion potential arising as a result of diffusion of the salt, which penetrated into the protoplasm along the fiber, the cation moving faster than the anion.

Previous studies on bioelectrical potentials have usually indicated that these diffusion potentials may be ignored because of their very small magnitudes, incomparable in value with the bioelectric ones. However, the possible role of diffusion potentials in the formation of the total salt electromotive force now needs to be revised, for the following reason.

Starting from the well-known formula of Henderson (1907) determining the value of the diffusion potential, it was shown theoretically and experimentally (Nasonov and Aleksandrov, 1934), that if two electrolytes (I and III) diffuse toward each other in a medium where still another electrolyte II is present, the total diffusion potential of the chain, electrolyte I-electrolyte II-electrolyte III will to a large extent depend on the concentration of the intermediate electrolyte II. The value of this electromotive force $E = A \ln \frac{B}{C_{II}}$, where A and B are constants and C_{II} the concentration of the intermediate electrolyte. This function has no limits; consequently by decreasing the concentration of the intermediate electrolyte C_{II}, through the solution of which the two electrolytes I and III diffuse each toward the other, the diffusion potential of the chain may be increased at will.

The membrane theory postulates that the majority of substances in the cell are present in the form of an ordinary aqueous solution. If this were so, then indeed the intracellular diffusion potential should have been very small (of the order of magnitude of tenths of a millivolt). It has already been stated that very few free electrolytes exist in living protoplasm, therefore electrolytes penetrating the cell and diffusing toward each other may develop considerable diffusion potentials, the magnitude of which cannot be ignored. These two possible sources of electromotive force cannot yet be differentiated exactly, but it is assumed that they both participate in the formation of salt currents**.

---

* The phase properties of living protoplasm explain very well the polarization of the latter on passage of direct current, and also its capacity properties.

** All that has been said on the role of diffusion potentials should be related to the above-mentioned experiments of Nernst with phase potentials (see Cremer, 1906).

Injury Potentials and Action Potentials

So-called injury potentials or resting currents will now be discussed. As already stated, they appear when a cut or partially injured part of the cell comes into contact with an intact surface by nonpolarizing (liquid) electrodes.

Figure 110B illustrates our concept of the cause of these potentials. In this figure an oblong cell cut in the right hand part (for example, a muscle fiber) is schematically described, the unshaded part representing the intact protoplasm. The shaded part is protoplasm injured as a result
185 of cutting, or by any other method. Two liquid nonpolarizing electrodes filled with isotonic NaCl are attached to both discontinuous and intact surface. After injury, the protoplasm loses its phase properties, and the electrolytes bound to proteins (mainly potassium phosphates) are released and pass into solution. The diffusion potential at the boundary of the injured sector and the liquid electrode is very small, since diffusion takes place in a medium rich in electrolytes (of the order of 0.1 millivolt). This can therefore be ignored.

Since protoplasm is a coacervate phase, a considerable potential spike could justifiably be expected at the boundary between the injured and intact protoplasm, where the phase, poor in electrolytes, comes into contact with the solution of potassium phosphates. Another spike occurs at the surface of contact between the liquid electrode and the uninjured protoplasm, here bathed in a NaCl solution. Both potential spikes are directed with their positive sign inside the protoplasm and the negative to the outside. Consequently, the connecting electrodes register the resting current equal to the difference between these two spikes. Since under otherwise equal conditions, potassium and phosphates bring about a greater negativity of protoplasm than does NaCl, this difference in potential in the injured part will be negative.

Comparing Figure 110B with Figure 109, it will be seen that the injury current is, strictly speaking, a salt current because a solution of sodium chloride is applied to one region of protoplasm, and potassium phosphate, which appears in the form of a free solution at the instant of injury to the protoplasm, to the other. This phase potential should be augmented by a diffusion potential as soon as the free potassium phosphate which has entered the intact protoplasm begins to diffuse along the fiber. This potential should be of considerable magnitude, since here diffusion takes place inside the protoplasm, which is poor in free electrolytes*.

---

* Chagovets (1909) at that time developed a theory according to which injury currents are a result of diffusion along the fiber of carbonic acid formed at the site of damage. His opponents maintained that the diffusion of carbonic acid should take place not only in the direction of the fiber but also in the opposite one, to the electrode, as a result of which the electromotive force will be equal to zero.

At that time it was accepted that all the electrolytes in protoplasm are dissolved and therefore the diffusion potential inside the cell should be equal to the potential directed to the electrode. But in fact this may not be so since protoplasm is now known to be poor in free electrolytes. That is why we consider Chagovets' ideas to be similar to ours.

186

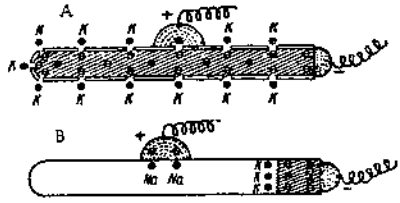


FIGURE 110. Scheme illustrating (A) the membrane and (B) the phase theories of formation of injury potentials (according to Nasonov and Aleksandrov, 1944)
For explanation see text.

Data were previously given which show that after excitation or injury of protoplasm, bound electrolytes are released. This alone should cause the appearance of a considerable diffusion potential (with a negative sign in the excitated section), since diffusion in the direction of resting protoplasm will occur in an electrolyte-poor medium.

It is also highly probable, although not proven, that the excited sector of the protoplasm loses its phase properties. Therefore, in the region of excitation, all conditions are present for the appearance of a phase potential. Thus we, as well as the supporters of the membrane theory, see features of great similarity in the phenomena of injury and excitation, and we consider the reasons for the appearance of action currents to be similar to those for resting currents.

The membrane theory of bioelectric potentials is now widely accepted by the majority of physiologists in the USSR and abroad. This theory will be briefly summarized. A comparative evaluation will then be given of a number of bioelectric phenomena in terms of the two theories, ours and that of the membrane concept.

The membrane theory in its classical form is based on the following assumptions: 1) all the electrolytes are present in the cell in the form of a free solution (Hill and Kupalov, 1930; Hill, 1935; Fenn, 1935, and others); 2) the cells are surrounded by a semipermeable membrane; anions do not penetrate at all through this membrane, while only those cations penetrate whose diameter (together with the solvate layer) does not exceed the diameter of the potassium ion; 3) after damage or excitation the membranes become permeable to all ions.

Figure 110 A illustrates Bernstein's membrane theory of injury currents (1912). The shaded part represents the cell content (protoplasm), being a free, aqueous solution of electrolytes, proteins and other substances. Only potassium, which is kept on the surface by nonpenetrating anions, giving the surface its positive charge, passes through the membrane pores. The areas shaded with dotted lines represent the nonpolarizing electrodes. The electrode connected to the site of the cut acts only as a conducting wire leading away the current. The diffusion potential arising here is relatively very small and it can be ignored. The membrane of the injured or excited sector loses its impermeability to ions, and the sector itself becomes electronegative in relation to the intact surface.

Common to both ours and Bernstein's theories is the fact that in both cases the appearance of electrical potentials in protoplasm is considered to be due to the presence of different ionic concentrations. However, to explain potentials of the order of tens of millivolts, Bernstein quotes the theory of membrane potentials, while we use the theory of phase potentials.

Careful comparison of the two schemes (Figure 110) discloses some basic differences. In the first place, the membrane theory assumes a
187 difference in potential formed in the resting cell. Connecting the

electrodes to the site of the cut, and to the intact surface, only closes the circuit.

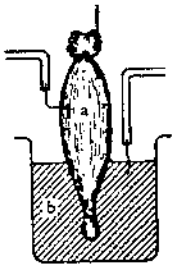


FIGURE 111. Experiment measuring potentials at the boundary between (a) muscle and (b) muscle mince.

According to our theory, the electromotive force arises only at the moment of injury or excitation, when the electrodes are released from their linkage with the protein substrate. In this respect our theory resembles the alteration theory of Hermann (1885), who assumed that at the site of alteration a rise in potential is due to chemical processes not clear to the author, but arising as a result of damage.

Another difference between the two theories is in the determination of the site of the potential spike. From the point of view of the membrane concept, this site is the membrane of the uninjured part of the cell. We, on the other hand, assume that the resulting electromotive force is the difference between the two spikes in potentials—at the boundary between the injured and intact sectors, and at the site of contact between the liquid electrode and the uninjured surface. This also differentiates our concept from that of Hermann, according to whom the spike of potential should occur only at the demarcation border.

Thus, in our opinion, the magnitude of injury potential is first of all determined by the electrolyte composition of the cellular fluid obtained on killing the protoplasm. This fluid, surrounding the intact protoplasm at the site of injury, gives it a negative charge.

Table 30

Difference in potentials between the part of the muscle surface immersed in a muscle mince, and the exposed part (according to Nasonov and Aleksandrov, 1944)

Time passed after immersion of the muscle in the mince	Potential (millivolts)
0 hr 0 min	18.9
0 " 25 "	28.1
0 " 50 "	28.4
1 " 20 "	33.8
1 " 30 "	33.9
One hour and 35 minutes later a cut was made and the injury potential measured.	39.2

What electrolytes are present in this fluid?

According to Fenn (1936), 83 out of 130 cation equivalents in the muscle fluid of the frog are attributed to potassium, and potassium is one of the cations most active in giving protoplasm a negative charge. Among these is the hydrogen ion, which is present at a higher or lower concentration in the cell fluid. From 82.6 anion equivalents of the muscle sap, 33.2

are phosphates, and these anions also belong to the negative charge range of the series discussed by us.

The fact that the muscle sap in contact with the intact surface can really cause a potential similar to the injury potential, is shown by the following experiment (Nasonov and Aleksandrov, 1944). A calf muscle of a frog was thoroughly minced and another muscle was immersed for one third of its length into this mince. The difference in potentials between the immersed part and the external surface was measured (Figure 111).

188 It follows from Table 30 that at first the difference in potentials increases slowly. This can be explained by the slow diffusion of cell fluid into the muscle. After 1½ hours it was measured as 34 millivolts. The muscle was then cut at the level of its immersion in the mince and the injury current measured. This was 39.2 millivolt. In other words, the potential between muscle and muscle mince almost reached the magnitude of injury current.

These are the general outlines of the phase and membrane theories. A number of phenomena may be explained by both theories. However, many data have recently accumulated which cannot be satisfactorily interpreted from the membrane theory aspect, but are well explained by our theory. In the following chapter these facts will be analyzed.

Chapter 2. Arguments in Favor of the Phase Theory

Site of the Potential Spike Occurring after Injury to the Protoplasm

According to the membrane theory, the potential spike noted in cells is localized in the uninjured part of the membrane between its inner and outer surfaces. Bringing about a contact between the uninjured surface of the cell and the site of injury by electrodes seems to connect the outer surface of the membrane with the inner one. At the site of injury proper, considerable potentials cannot arise, since the membrane theory considers the cell content to be a simple aqueous solution.

According to the phase theory, the difference in potentials observed in the damaged cell is composed of two spikes: one at the intact surface, as a result of unequal solubility in the protoplasm of Na and Cl from the surrounding liquid, and the other at the boundary between the injured and the uninjured cell parts, as a result of unequal solubility in protoplasm of cations and anions of released electrolytes. In addition, there are reasons to believe that these potentials are also augmented by a diffusion potential, arising as a result of an unequal diffusion rate of released cations and anions inside protoplasm.

As a confirmation of the validity of the membrane theory, the old experiments of Hermann (1871) are sometimes cited, showing that temperature does not affect the injury current of a cross section of muscle, whereas heating or cooling the uninjured part causes a considerable change in magnitude of the potential.

Similar experiments by Verzar (1911) on nerve tissues contradict these observations. He showed that temperature also affects the resting current when applied to injured and uninjured segments. However, the

author, together with Bernstein, cites the membrane theory in showing that in nerve fibers the nodes of Ranvier play the role of cross membranes dividing the nerve fiber into separate segments*. Later, Pauli and Matula (1916) repeated Hermann's experiment in a very thorough study on frog muscles. They came to the conclusion that here, exactly as in the nerve, 189 the magnitude of resting current changed considerably on heating both injured and uninjured parts. In addition, these authors succeeded in revealing Hermann's source of error. Bernstein (1916, 1917) attempted to dispute on this point with Pauli and Matula but could not master any convincing proof against their data.

Thus, data on the effect of temperature on resting currents effectively negate the membrane theory, but are nicely explained by the phase theory, insofar as the thermal effect is seen both on the injured and intact segments of tissue. The experiments of Krouse and Burge (1936) also localize the spikes of potentials to the region of injury. These authors cancelled the negativity of a cross section of frog calf muscle by placing a drop of $CaCl_2$ solution on the wound. Subsequent application of H_3PO_4 or Na_2HPO_4 restored the difference in potential. The authors presumed that the reason for appearance of electromotive force was the anion of phosphoric acid released during excitation or injury, following decomposition of creatine phosphate and adenyl pyrophosphate. Calcium chloride precipitates phosphates and, according to the authors, by doing so removes the cause of negativity of the injured sector. This concept resembles ours, and no matter what interpretation is given to these interesting experiments, it must be admitted that they clearly contradict the membrane theory, according to which the site of the cut is only a conductor leading away the current, while the electromotive force is localized at the surface of the uninjured membrane.

Steinbach (1933), using Pecten muscles, showed that application of different electrolytes to the injured part of the cell affects the resting current much more than the same treatment when applied to the uninjured surface. This also contradicts the membrane theory.

It has been known for a long time that on washing any part of the muscle or nerve surface with dilute Ringer's solution or distilled water, this part becomes electropositive in relation to the intact part. This fact cannot be explained by the membrane theory, which is based on complete impermeability of the membrane to Na and Cl. Indeed, if sodium does not penetrate the membrane, then changing its concentration at any point of the surface should not affect the magnitude of the potential, excluding those cases where the change is so extensive that it loosens or damages the membrane. Only in the latter circumstances could this sector be expected to become negative. However, dilute Ringer's solutions turn the surface positive (Oker-Blom, 1901; Sugi, 1935; Fenn, 1931). These data were also confirmed by our experiments. We maintain that the electromotive force of the injury current consists of two spikes of potential, at the cut and at the intact surface. The latter is due to the greater solubility of sodium in protoplasm as compared with Cl. Consequently, NaCl turns the cell surface negative (Figure 110 B), and its removal from the solution should do the reverse. Somewhat sceptically, Fenn concludes (regarding the possibility of explaining these facts by the membrane theory:) "In any

---

* Nowadays this viewpoint would be totally unacceptable.

case these positive water potentials were not sufficiently considered in the past. If they really mean elimination of the sodium potentials (with negativity on the outside) then this will force us to seriously change the existing
190 theories" (Fenn, 1936, p.466). Such an admission coming from an ardent supporter of the membrane theory really indicates that this theory is undergoing a serious crisis.

Increase in the "Lowered Injury Potential" on Renewal of the Cut

It has been known for a long time that after injury the resting current decreases with time. This phenomenon was studied in detail and in various tissues by Engelmann (1877). The author showed that in certain cases, on infliction of a new cut at a small distance from the old one, a spike is seen in the lowered potential. Bernstein (1912) attempted to use these experiments to confirm the localization of the spike of potential in the membrane. According to the membrane theory, the fall in potential after traumatization may have occurred either as a result of gradual dying of the entire cell, accompanied by an increase in permeability of all sectors of its membrane, or as a result of gradual exit of protoplasm electrolytes through the point of injury to the cell. In both cases, renewal of the wound should have given no effect.

A third possibility may be assumed, namely, regeneration of the membrane at the site of the cut. This process should also cause gradual decrease in the potential difference, but then on renewal of the wound an increase may be rightly expected in this difference. In addition, on renewal of the damage, increase in lowered potential may arise where the resting current is eliminated, not from one cell but from a series of cellular elements following each other.

According to the phase theory the cause of the electromotive force is the amount of free electrolytes released at the site of trauma. These electrolytes gradually diffuse into the surrounding Ringer's solution and intercellular space, and are replaced by sodium chloride. In our opinion, this is the reason for the fall in potential over a period of time. On renewal of the wound, a new portion of electrolytes is released, due to which the difference in potentials again increases.

Studying various tissues, Engelmann observed that with time the potentials of injury in nerve and heart muscle decreased rapidly and that they showed a marked increase after another wound. In skeletal muscles, however, under the same circumstances the injury currents showed a slight increase, which decreased comparatively slowly with time. Following Engelmann, Bernstein explained this difference by the fact that the nerve fiber consists of segments 0.5 mm long, divided by nodes of Ranvier. According to Bernstein, each such segment behaved as a functionally independent cell and therefore the damage inflicted upon the nerve did not spread beyond the first node of Ranvier. Subsequently, the nerve died simultaneously along its entire length. In the heart muscle the role of separate but consecutive cells is played by the segments between the so-called inserted, or intermediate plates. The muscle fiber may thus be considered as one cell. Damage inflicted on muscle spreads along the fiber

to its very end. Here the current falls gradually and is never reinforced
191 on renewal of damage.

At first sight, all the facts would seem to be well explained by the membrane theory. Nevertheless, hardly anyone now will seriously maintain that the segment of the axon between two nodes of Ranvier may be considered as a separate cell. The same may also be said of the segments of the fibers of heart muscle. More than that, Bernstein himself, in a footnote to his book (in small type, page 107) remarks that in the olfactory nerve—in which there are no nodes of Ranvier—instantaneous restoration of the potential, lowered with time after renewal of the wound, is observed. In order to integrate this fact with the requirements of the membrane theory, further studies are necessary, in the author's opinion.

Concerning skeletal muscles, Bernstein allowed himself an inaccuracy in quoting Engelmann's data, ascribing to him the statement that in this tissue, renewal of the wound never caused an increase in the resting current. However, from the reports given by Engelmann (1877), it is clearly evident that on renewal after a short time period (1 hour), an increase in potential was not observed. Such an increase is undoubtedly observed after 24 hours and is very weak after 48 hours. According to the membrane theory, however, this should not take place at all in muscles.

In view of the fact that all data connected with the localization of the increase in potential are of paramount importance for the applicability of this or another theory, a large series of experiments was conducted by us.

First of all, by the method of in situ microscopic observation, and also by the use of "time-stop"* photomicrography, the process of decomposition of muscle fibers was studied in detail under different conditions, using skeletal muscles of the frog (Nasonov and Rozental', 1947; S.N. Aleksandrov, 1948a, 1948b, 1949; Raevskaya, 1948; Gramenitskii, 1949; Aleksandrov and Leushina, 1953; Leushina and Aleksandrov, 1953), muscles of insect appendages (Nasonov and Rozental', 1947) and muscles from the abdomen of fresh-water crayfish (Gramenitskii, 1948).

In agreement with the previous data of Engelmann (1877), a fundamental difference was observed between the processes taking place at the site of the cut skeletal muscle fibers and fibers of the heart muscle and nerve. In the former, the boundary of the damaged protoplasm was never localized at the same point. The zone of damage spread gradually along the fiber, involving new segments of undamaged protoplasm all the time (Figure 112) as if the dying protoplasm itself were a cause of death of adjacent healthy segments.

According to Raevskaya, the initial speed of irradiation of injury along muscle fibers in frogs is 0.48 mm per hour. According to Gramenitskii this speed is 0.65 mm per hour in the flexor muscles of the tail of the fresh-water crayfish.

The most important fact is that the process of irradiation of damage in skeletal muscles absolutely excludes the possibility of regeneration of the boundary membrane, first because there is no sharp border between the injured and intact protoplasm, and secondly, because the whole zone of injury moves constantly along the fiber. Consequently, there is no op-
192 portunity for the boundary membrane to be repaired.

---

* [This probably means: continuous photomicrography, where the time element can be determined from the reel].

Quite a different result is obtained on cutting heart muscle fibers. In our laboratory, Gramenitskii studied a live preparation of frog heart. Separate muscle fibers of the auricle were cut, followed by microscopic observations for 24 hours and "time-stop" photomicrography of the pulsating heart. Immediately after cutting, a swelling formed at the end of the fiber. This refracted light intensely and stained with vital stains, from which a zone of turbidity spread for a small distance, gradually passing into normal protoplasm. The dimensions of the whole damaged segment were approximately 180 μ. Measurements performed after 24 hours on each separate fiber gave exactly the same number as those obtained immediately after cutting. Consequently, no irradiation of damage took place here.

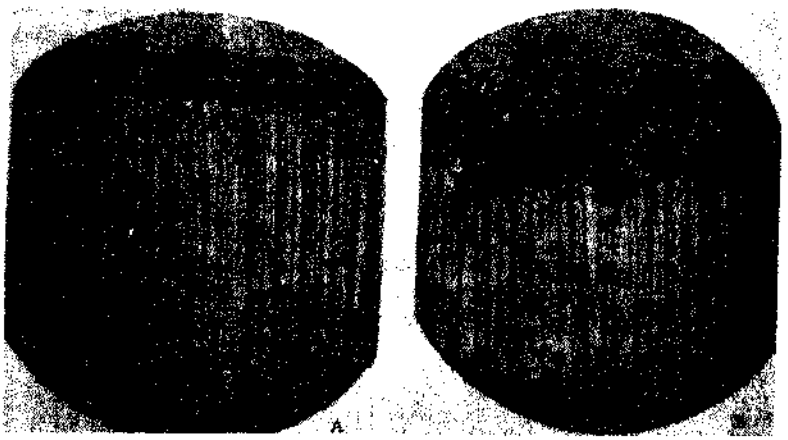


FIGURE 112. Decomposition of cut pectoral muscles in frog, in Ringer's solution (according to Raevskaya, 1948)

A—30 minutes after cutting; zone of nodular contractions at edge of cut comprises 0.16 mm. B—same muscle 60 minutes after cutting; zone of decomposition (in the form of nodular contractions) comprises 0.41 mm; decomposition in different fibers progresses at different levels.

In our laboratory a similar picture was also observed by Romanova, using dark field illumination of an isolated nerve fiber of a nerve-muscle preparation of the frog. The intactness of the fiber was previously checked by its ability to conduct impulses. An axon of an undamaged fiber is optically empty in the dark field. Immediately after cutting, the injured part became luminous, and subsequently, in the direction away from the site of the cut, luminous grains appeared, the number of which gradually increased. This process, however, was rapidly halted, and in spite of the data of Engelmann (1877), not necessarily at the boundary of the node of Ranvier. Subsequently, there was no irradiation of damage and the pre-
193 paration gradually died along its entire length. The same results were obtained in studying nerve preparations stained with methylene blue.

This is the morphological picture of damage on cutting fibers of heart muscle and nerve. These tissues differ from skeletal muscle by the lack of

irradiation of damage. Correspondingly, there are two different types of curves for changes in injury potentials in relation to time.

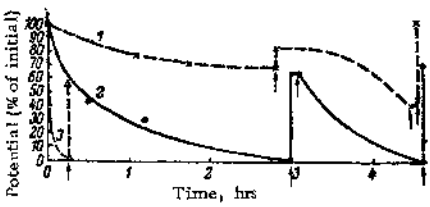


FIGURE 113. Decrease in injury potential and the effect of renewal of cuts on (1) the sartorius muscle, (2) the sciatic nerve and (3) heart muscle of frog (according to Nasonov and Aleksandrov, 1944). Times of the renewal of cuts are designated by arrows.

Figure 113 shows typical curves of a decrease in potential difference of injured muscle (sartorius muscle), nerve (sciatic nerve) and heart muscle of the frog (effect of time and renewal of wound). All three tissues were kept in Ringer's solution (at 18° C) between measurements. The curves show that the potentials of heart muscle and of the nerve decreased rapidly, and again increased considerably, on renewal of the wound. Injury did not irradiate in these tissues, and the rate of fall in potential was determined by exit of electrolytes from the small nonincreasing segment of injury. In the skeletal muscle the potential decreased considerably slower, and the effect of renewal of wounds was not as great, but undoubtedly existed. Here injury spread along the fiber, causing continuous release of new groups of electrolytes. That is why, in spite of constant exit of electrolytes, the decrease in potential took place relatively slowly.

Different Rates of Decrease in Injury Potential in Ringer's Solution and in the Moist Chamber

If the true reason for the decrease in injury potential was really the exit of salts from the wound, then it might be expected that in the nerve, a faster decrease would occur in Ringer's solution than in the moist chamber. The experiment confirmed this assumption.

Figure 114 shows that in Ringer's solution, after one hour and 35 minutes, the difference in potentials decreased to zero, while in the moist
194 chamber the nerve clearly showed this phenomenon after only 17 hours. At the same time, in both cases the nerve remained alive, as is shown on renewal of the wound.

However, these experiments can be criticized by claiming that the conditions of nerve in Ringer's solution differ from those in the moist chamber, and that these differences, for reasons still unknown, affect the rate of decrease in potential. In similar experiments performed with muscles, this objection was eliminated in the following manner (Nasonov and Aleksandrov, 1950). The wide ends of twin sartorius muscles of the frog were cut off, and the differences in potential between the injured and intact parts (first cut) were measured at once. Both muscles were suspended in a vessel with Ringer's solution in such a manner that one of them touched the liquid with its cut end, allowing the constant exit of salts from the site of the cut. The twin muscle, as seen from Figure 115, was suspended alongside at a distance of 2 mm from the surface of Ringer's solution.

After 3 hours, the differences in potentials (which decreased during that time) were again measured.

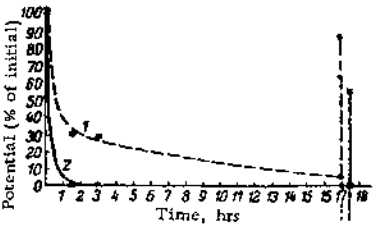


FIGURE 114. Decrease in injury potentials of the sciatic nerve of the frog in (1) moist chamber and (2) in Ringer's solution (according to Nasonov and Aleksandrov, 1944). Legend as in Figure 113.

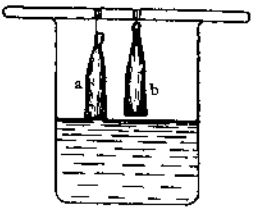


FIGURE 115. Experimental method of measuring injury potentials in muscles (according to Nasonov and Aleksandrov, 1950)

a—wound surface of muscle in contact with Ringer's solution; b—muscle not in contact with Ringer's solution.

Table 31

Changes in injury potential of muscles on renewal of cuts; under conditions of a moist chamber, and on contact of the cut with Ringer's solution (according to Nasonov and Aleksandrov, 1950)

Washed slices							Unwashed slices						
Muscle no.	Potential of the 1st cut		% of drop	Potential of 2nd cut	% increase	Potential of 2nd cut (% of initial)	Muscle no.	Potential of the 1st cut		% of drop	Potential of 2nd cut	% increase	Potential of 2nd cut (% of initial)
	immediately	after 3 hours						immediately	after 3 hours				
1	39.0	29.5	−24	32.5	+ 10	83	1	36.0	27.0	−25	30.0	+11	83
2	40.5	18.5	−54	30.0	+ 62	74	2	53.0	34.0	−36	36.0	+ 6	68
3	48.0	30.0	−37	38.0	+ 27	79	3	43.5	29.0	−33	32.5	+10	75
4	37.0	18.5	−50	31.5	+ 70	85	4	49.0	38.5	−21	41.5	+ 8	85
5	27.0	13.0	−52	37.5	+188	139	5	45.0	34.5	−24	40.0	+16	89
6	51.0	24.5	−52	37.5	+ 53	74	6	53.0	42.5	−20	43.5	+ 2	82
Average			−45		+ 68	89	Average			−26		+ 9	80

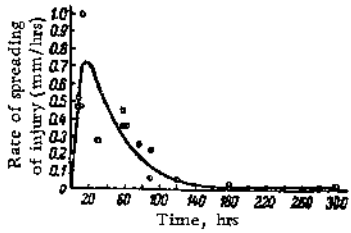


FIGURE 116. Change in rate of spread of injury in sartorius muscle of the frog (according to Raevskaya, 1948)

It follows from the data in Table 31, that the difference in potential in the muscles which were in contact with the liquid decreased by an average of 45%, while in those not in contact with the liquid—only by 26%. Subsequently, a new cut was made 3 mm distant from the old wound. A very clear-cut result was obtained: in the muscles touching the liquid, the difference in potentials increased on the average by 68%, as compared with the previous measurement. In muscles not touching the solution, a very small increase was observed—9%. As a result, after the second cut similar differences in potentials were obtained in both cases (89% and 80%). Thus, the difference in magnitude of effects of renewal obtained in these two variations depended mainly on the extent of the decrease in the difference in potentials during the time preceding the renewal of the wound. These experiments seem to confirm the assumption that the main reason for the appearance of bioelectrical potentials were the electrolytes released at the site of injury or excitation.

However, on the basis of these considerations the question may arise: why, after all, is a fall in potential seen in skeletal muscles? If irradiation
195 of damage secures continuous release of new portions of electrolytes, then it could be rightly expected that a certain constant level of the potential might be established. The magnitude of this level should be determined by the constant concentration of electrolytes at the demarcation border, as a result of equilibrium between the processes of their entry and exit. However, this would be so only under the condition that the rate of spread of damage remained strictly the same. On slowing down of irradiation, the difference in potentials should decrease, and vice versa. Raevskaya (1948), studied in detail the rates of spread of damage under different conditions. It was observed that immediately after cutting, this rate was quite high and might even have increased during the first few minutes. Subsequently, however, slowing down of irradiation was observed, continuing for 3 to 4 hours after traumatization (Figure 116). In muscles, a constant suppression of irradiation thus occurred, as a result of which the injury potential should have slowly decreased. Renewal of the wound again accelerated the process, causing a spike in the potential.

Thus, all the data cited in the present chapter unconditionally confirm the localization of the potential of injury, not only on the intact surface of the cell but also at the site of the cut itself. Experiments on the effect of temperature, the positive potential from water, the effect of application
196 of electrolytes to the cut, and finally, the data on strengthening the lowered potential upon renewal of the cut, support this hypothesis. It may be considered an established fact that, in spite of Bernstein's statements, a sharp increase in potential occurs on renewal of the wound, not only in nerves and heart muscle but also in skeletal muscles. The constant irradiation of damage along the fibers in the latter excludes the possibility of regeneration of a new membrane, i.e., the possibility of explaining this phenomenon from the point of view of the membrane theory.

Increase in Injury Potential of Muscles After Cutting

The question of latent periods with resting currents played an important role in the debate on the nature of injury potentials in cells. If the difference in potentials in cells is localized only at the undamaged surface, it should be detected immediately after injury to any part of the membrane. If, however, the electromotive force arises after injury at the site of a wound, a certain lapse of time may be necessary for its development. The studies of Hermann (1877), Garten (1901, 1904), Bernstein and Tschermak (1904) were devoted to this problem. However, Bernstein and Tschermak themselves stated that their studies lacked sufficient data to solve the problem of preexistence of potentials. Indeed, one cannot disagree with Bernstein (1906), that the impossibility of detecting the latent period by existing methods still does not prove the preexistence of potentials, since the rate of their appearance after injury may be of the order of speeds of molecular reactions. At the same time, Garten (1904), described a gradual increase in potential lasting up to 5 msec, which could be explained, according to the membrane theory, by the influence of gradual pressure of the edge of the cutting instrument on the living cell. In any case, these studies proved that the potential of injury arises at a speed of only fractions of a millisecond.

In addition to the instantaneous rise in potential on traumatization, as described above, a relatively slow increase in injury currents was subsequently observed in muscles. This reached a maximum after infliction of the wound, followed by a decrease in potential, characteristic for all tissues. This phenomenon was first described by Pauli and Matula (1916) and later by Sugi (1935), who evidently did not know of the work of the former authors, since he did not refer to them. Pauli and Matula observed an increase in injury currents in the sartorius muscle of the frog inside a moist chamber, lasting from 15-20 minutes to 1 hour. In liquid paraffin this increase lasted 30 minutes, reaching 20% of the initial value. In Sugi's experiments with the same tissue, the maximal increase was observed 10 seconds to several minutes after cutting, this period being prolonged if the muscle was not sufficiently fresh.

The phenomenon of increased potential in the sartorius muscle of the frog was studied in detail by Nasonov and Aleksandrov (1944, p. 19). The experiments were performed in January and February on winter frogs kept at 10° C-13° C. Altogether, more than 50 muscles were examined. They all showed an initial increase in electromotive force, subsequently replaced by a slow decrease.

197 From analysis of the data of Nasonov and Aleksandrov (1944, p. 21), it is evident that the average value of increase in potential in 80 experiments was 4.8 m/volts, while the average time necessary to reach the maximum was 7.8 minutes. However, these values were not very convincing since in isolated cases considerable deviations were observed and the course of the curves was very erratic. The maximum increase of voltage was 14.3 millivolt, sometimes attained after 30 minutes, the increase in potential comprising 44% of the initial value. Experiments performed on frog calf muscles gave similar results, but here the value of the maxima was less than in the previous case. In order to check whether the observed increase in potentials was associated with changes in the electrode wires, experiments were performed in which the surface of the cut muscle was brought

into direct contact with Ringer's solution. The result was exactly the same.

Thus, the increase in injury potential on cutting tissue (reaching 44% of the initial one) is beyond doubt.

How can this be explained by the membrane theory?

If, as required by the membrane theory, the electromotive force is really localized only at the intact surface of the membrane, this phenomenon might be explained by assuming that due to cutting at the end of the muscle the permeability of the entire membrane is instantaneously increased and subsequently begins to decrease back to normal. In order to check this point, the following series of experiments was performed.

The tip of the sartorius muscle of a frog was cut off, and electrode wires were placed firstly on the wound (Figure 117 A, 1) and secondly in the middle of the uninjured surface (Figure 117 A, 2). The muscles were kept in a moist chamber, and as usual, the difference in potential was measured
198 every 1-3 minutes. The time interval between cutting and first measurement was reduced to 25-30 seconds. The electromotive force curve rose at the beginning and after reaching a maximum value, subsequently began to decrease. At that moment, the opposite tip of the muscle was cut off without moving the electrodes 1 and 2 and it was attached to the wire of electrode 3. Electrodes 1-3 were connected by a bridge which subsequently enabled measurements to be made of the difference in potentials between the common point of the uninjured surface (electrode 2), and two cuts made at different times. Figure 117 A shows that the injury potential of the first cut (a) increased from 37 to 42.6 millivolts, after which it began to decrease rapidly. At that moment a second cut was made at the opposite end, the potential of which (b) increased simultaneously with the continuing drop in the first one, in spite of the fact that in both curves the electrode of the uninjured sector was the same common one. A similar picture is seen in Figure 117 B.

It is self-evident that in this common sector there could not have occurred a simultaneous increase and decrease in the permeability of the membrane of the intact surface, as would have to be assumed on the basis of the membrane theory. The increase and decrease in potentials at opposite ends of the muscle cannot be explained by diffusion phenomena in the aqueous medium, because the increases in electromotive force were too great (up to 14 millivolt) for the diffusion potentials in an aqueous solution rich in electrolytes. The possibility of explaining these phenomena as a result of changing resistance in the two opposite sectors of the muscle must be rejected, since the measurements in these sectors were performed by the compensation method. We cannot imagine any possible explanation
199 of these experiments by the membrane theory without using artificial conjectures.

In our opinion the value of the muscle potential at any moment should depend on two factors. First, on irradiation of injury, when new portions of electrolytes are released all the time, a fact which should raise the curve of potential. Second, on the constant exit of electrolytes from the region of the cut, which causes a fall in this curve. As a result of interaction of these factors, a curve of any shape may be obtained. As already stated, according to Raevskaya, the rate of spread of damage along the fiber was uneven: at the very beginning it increased for a short time, after which it decreased (Figure 116).

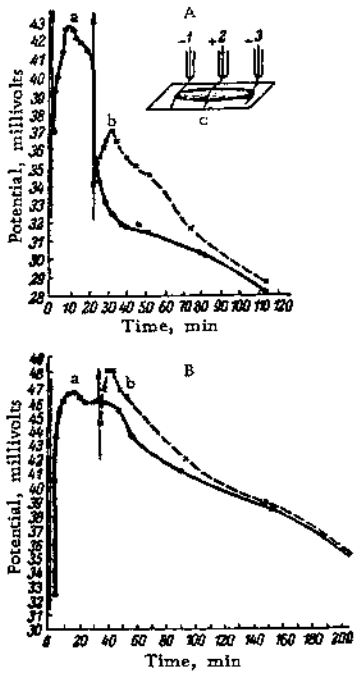


FIGURE 117. Change in injury potentials of two opposite ends (a and b) of the sartorius muscle of a frog; in two different experiments (A and B) (according to Nasonov and Aleksandrov, 1944)

c—diagram of the experiment.

If the maximum potential really depended on the uneven course of the process of irradiation of injury and degeneration of living protoplasm, then in the nerve fiber, where this process ceased very rapidly in the region of the cut, the maximum, which was so characteristic for muscles, could also not be detected. Experiments performed by the same method as with muscles confirmed this assumption (Figure 118).

Thus, increase in injury potential in muscles after inflicting a cut is not explicable by the membrane theory, but is easily understood if the appearance of the potential is shown to be due to electrolytes released by protoplasm in the region of the cut.

Relationship between the Method of Cutting and the Magnitude of Injury Potential

The magnitude and stability of injury potentials depend on the method of inflicting the cut. The potentials obtained were more stable if the end of the nerve where the cut was performed was crushed, pressed or killed by a solution of KCl. With a simple cut by a sharp razor or scissors, the difference in potential was lower, and disappeared rapidly. This cannot be understood according to the membrane theory, since according to it the site of the cut is only an electrode leading away the current, recording the preformed difference in potentials between the external and internal parts of the undamaged membrane. However, according to the phase theory this should really be so, because the injury potential is determined by the fraction of free electrolytes released from damaged protoplasm. Therefore the magnitude and stability of the potential should depend both on the extent of damage and on the dimensions of the damaged sector. In this respect the work of Makarov (1949), who determined the relationship between injury currents and speed of infliction of the cut, is of great interest. He constructed an instrument with which, by lowering a sharp razor, it was possible to vary the time of dissection of the frog nerve (nerve-muscle preparation) from 28 to 150 msec.

Table 32, compiled according to his data, shows that the magnitude of injury potential is inversely proportional to the speed of cutting. The more slowly the edge of the razor passes through the nerve, the larger will be the sector of adjacent tissue damaged, and vice versa. This can be compared with the effect of a rapidly flying bullet which passes through glass,

leaving only a small hole, while the adjacent sectors of the glass may remain completely undamaged. However, the same bullet when moving at a lower velocity will completely break the whole glass.

It is interesting that in Makarov's experiments, rapid cuttings of the nerve (30-40 μ sec) caused no contraction of muscle, because under these conditions a spreading impulse did not arise. Obviously the magnitude of injury current was below the threshold of excitation, spreading without decrement. The results of Makarov's experiments are in agreement with the phase theory but not with the membrane theory.

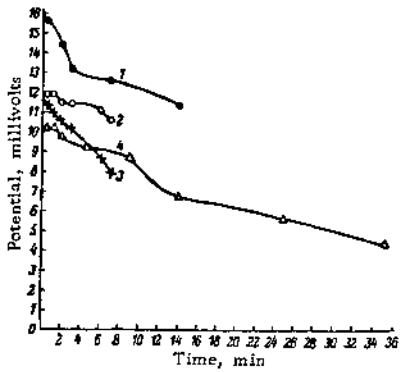


FIGURE 118. Examples of change in magnitude of injury potential in sciatic nerves of a frog (1-4) (according to Nasonov and Aleksandrov, 1944)

Injury Potential Exceeded by Action Potential

If the difference in potential between the cell content and its external surface is indeed predetermined by the concentrations of electrolytes dissolved in its protoplasm, as postulated by the membrane theory, then the maximum value of this difference will be obtained when the cut surface of the cell is connected with the uninjured one by nonpolarizing electrodes. Thus action currents can under no circumstances be greater than resting currents. This hypothesis, an absolute corollary of the membrane theory, was formulated for the first time by Bernstein himself: "As a consequence of this theory one would have to assume that the maximal limit of negative fluctuation should be the membrane potential, and it should not acquire the opposite charge upon excitation*".

Bernstein stated that while working with muscles, he never observed the action current to exceed the magnitude of resting current. Burdon-Sanderson and Gotch (1891) nevertheless obtained an action current of 100 millivolt on the muscle, while the maximal resting current did not exceed 80 millivolts. However, the authors did not determine these two values simultaneously. According to Bernstein, it is possible that in these studies the resting current decreased rapidly and at the time of measurement was no longer maximal. Bernstein himself observed that in nerves the negative fluctuation upon excitation can considerably exceed the injury current. However, the author explains this fact by division of the nerve fiber, at the points of the nodes of Ranvier, into a number of segments, as a result of which the magnitude of injury potential may, from the very beginning, be lower than that of the current of action.

---

* "Eine Konsequenz dieser Theorie würde nun sein, dass die negative Schwankung eine maximale Grenze erreichen müsste, welche durch die Stärke des Membranpotentials gegeben wäre, und dieses bei der Reizung sich nicht umkehren könnte" (Bernstein, 1912, s. 105).

Table 32

Relationship between the magnitude of injury potentials of a frog nerve, and the speed of cross-section of the nerve (according to Makarov, 1949)

Time of cross-section of the nerve (in seconds)	Current of injury (millivolts)	Appearance of transmitted excitation (contraction of muscle)
33	8.6	—
38	6.1	—
44	14.2	—
100	19.0	+
150	30.0	+

Note: In those cases where Makarov cites several experiments, arithmetic means of his results are given in the present table.

This concept, supported in the last century by Engelmann and others,
201 should now be considered as arbitrary and unfounded. The obvious incompatibility of facts with requirements of the membrane theory was already known in the last century and caused considerable problems to the formulator of the theory himself—Bernstein (1912). Later, however, these inconsistencies were somehow forgotten, and 27 years later, after Hodgkin and Huxley (1939), by using a new tissue, the giant isolated nerve fiber of Loligo (squid), reproduced the data of Burdon-Sanderson and Gotch (1891), these inconsistencies were again stated so clearly that the old explanations were no longer valid. First, this tissue has no nodes of Ranvier since the nerve is nonmedullated. Further, one of the recording electrodes was introduced inside the nerve fiber, while the other was placed externally on the undamaged surface, thus, according to the authors, enabling direct measurement of the transmembrane increase in potential, which was 50 millivolts*. On passage of the impulse and the assumed complete depolarization of the membrane, it might have been expected that this difference in potential became zero. In reality, however, the pointer of the galvanometer not only reached zero, but deflected to the opposite side, since the excited region was more negative than the site of the cut by 45 millivolts.

This study, performed with great experimental virtuosity, and faultless in its methodology, astounded physiologists. The membrane theory was subjected to critical review (Rubinshtein, 1949a, 1949b). A number of papers appeared in which action currents and resting currents were compared, using various tissues. Table 33 gives the results of these studies.

From these data it follows that in various animals (mollusks, arthropods, amphibians, mammals) the action current in nonmedullated and

---

* In reality the authors, by introducing an electrode inside the fiber, could not help causing mechanical damage to the protoplasm, thus eliminating the ordinary injury potential. The possibility of short-circuiting through the intercellular spaces was excluded in these experiments.

medullated nerves exceeds the resting current 1.6-2.0 times; in skeletal and heart muscles and in Purkinje's fibers of the heart it was exceeded 1.3-1.4 times.

Table 33

Magnitude of action and resting potential of various tissues, (according to Troshin, 1956)

Animal	Tissue	Current (millivolts)		Ratio between current of action and current of rest	Authors
		of rest	of action		
Loligo forbesi	Nonmedullated nerve	50	90	1.8	Hodgkin and Huxley, 1939.
" "	The same	48	88	1.8	Hodgkin and Katz, 1949.
L. pealii	" "	51	104	2.0	Curtis and Cole, 1942.
Sepia officinalis	" "	62	120	2.0	Weidmann, 1951.
" "	" "	—	124	—	
Homarus vulgaris	" "	—	110	—	Hodgkin and Rushton, 1946.
" "	" "	62	106	1.7	Hodgkin and Huxley, 1945.
Carcinus maenas	" "	—	116	—	Hodgkin, 1947.
" "	" "	71-96	116-153	1.6	Hodgkin and Huxley, 1945.
Rana esculenta	Sciatic nerve	71	116	1.6	Huxley and Stämpfli, 1951.
R. temporaria	Skeletal muscle	88	119	1.4	Nastuk and Hodgkin, 1950.
R. pipiens	Heart muscle	50-90	65-115	1.3	Woodbury and others, 1950.
Canis familiaris	Purkinje's fibers of the heart	90	121	1.3	Draper and Weidmann, 1951.
Capra hireus	The same	94	135	1.4	

That the action current exceeds the resting current was also observed by Graham and Gerard (1946), using isolated fibers of skeletal frog muscles; by Woodbury et al. (1951) on isolated muscle fibers of frog heart; by Fing et al. (1951) on embryonic muscle fibers of the chick; by Woodbury (1952) on isolated nerve fibers of the sciatic nerve of the frog, and by Trautwein, Zink and Kayser (1953) on isolated fibers of skeletal muscles of warm-blooded animals. No one doubts any longer the fact that the action potential exceeds the resting potential, but it is very difficult to explain this fact in terms of the membrane theory.

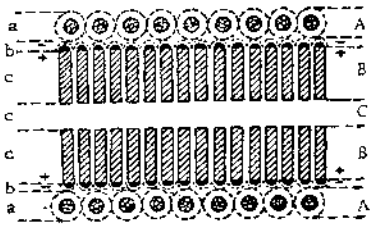


FIGURE 119. Scheme of structure of semipermeable membrane, according to Danielli and Harvey (from Höber, 1945)

202

A—a layer of globular proteins; B—a layer of oriented lipoid molecules; C—nonoriented layer of lipoid molecules; a—hydrated protein molecules; b—ionized ends of lipoid molecules; c—nonionized ends of lipoid molecules; a+b—hydrophilic zone; C+c—lipophilic zone.

According to this, the surface of the membrane depolarizes after excitation, becoming electronegative in relation to the resting surface. This was usually explained by the loss of selective ionic permeability of the membrane. But in that case, on connecting the cross-cut with the excited surface no difference in potentials should have been obtained, and if it was, electronegativity would appear at the cut (provided depolarization was incomplete on excitation). In reality, however, a considerable difference in potentials is obtained with electropositivity at the cut. The excited surface did not simply lose its positive charge but acquired a negative charge. How could this fact be explained?

Numerous hypotheses were brought forward to explain this phenomenon, all highly artificial and none which could be considered even partially satisfactory. This is because, without exception, the authors, instead of abandoning the obviously unsuitable membrane theory and seeking other explanations, tried to modernize this theory by complicated and arbitrary superstructures in order to adapt it to the discordant facts.

It is not necessary to discuss here all the theories which have been put forward on this subject. Those interested are referred to the papers by Grundfest (1947) and Hodgkin (1951). A few extracts which, in our opinion, are more fully elaborated will be considered.

Hodgkin and Katz (1949) started from the classical assumption that the main electrolytes of the protoplasm exist in a free aqueous solution. According to them, the magnitude of the resting potential in a nerve fiber can be calculated from the formula

$$E = \frac{RT}{F} \ln \frac{P_K(K)_i + P_{Na}(Na)_i + P_{Cl}(Cl)_0}{P_K(K)_0 + P_{Na}(Na)_0 + P_{Cl}(Cl)_i},$$

203 where P_K, P_{Na}, P_{Cl} are the permeability constants for potassium, sodium and chlorine, (i.e., diffusion rates across the membrane). The concentrations inside the fiber (i) and on the outside (O) are given in parentheses.

If from this formula the magnitude of the resting current for an isolated fiber of Loligo is calculated, assuming that $P_K:P_{Na}:P_{Cl} = 1.0:0.04:0.45$ then the value of 59.5 millivolts is obtained, which is close to the experimental one (61–62 millivolts). In order to justify the magnitude of the observed action potential (38 millivolts above that of the resting potential), it is necessary, in the authors' opinion, to assume that in relation to potassium and chlorine the permeability of the membrane on excitation remains the same ($P_K = 1.0$, $P_{Cl} = 0.45$), while for sodium it increases 500-fold and becomes 20.0.

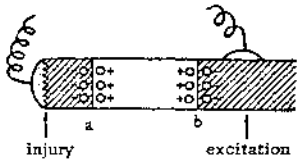


FIGURE 120. Scheme illustrating the relationship between currents of rest and of excitation, in terms of the phase theory of biopotentials

a—division boundary between intact and damaged protoplasm; b—the same, between the intact and excited protoplasm.

But how can this occur?

The authors do not give a satisfactory answer to this question. Incidentally, they produce a hypothesis that at the time of excitation, dehydration of ions may take place, sodium becoming more mobile than potassium. But this new assumption is entirely arbitrary, and itself requires explanation. In addition, it is known that on excitation considerable amounts of potassium leave the cells. This fact contradicts the authors' postulate that permeability of the membrane to potassium should not increase.

Danielli (1941) developed yet another hypothesis. He assumed that the membrane consists of two rows of lipid molecules, each of which turns with its hydrophilic negative ends to the outside, and the positive hydrophobic ones to the inside (Figure 119).

This system is electroneutral. On excitation, the inner layer of lipoid molecules becomes disoriented, and the surface of the cell becomes electronegative. After the excitation wave passes, the lipoid molecules again become arranged in the form of a palisade and the surface again becomes electroneutral. At the Gagra Conference, Rubinshstein (1949b), the most orthodox supporter of the membrane theory in the USSR, defended this hypothesis as the most tenable.

The speculative and arbitrary nature of this hypothesis will be seen at once. It is not comprehensible what forces cause the lipoid molecules to be arranged in the form of films, nor what makes the water-insoluble lipoid accumulate in the form of droplets, which is most likely from the thermodynamic point of view. We know that orientated monolayers of molecules may form at the boundary between two phases. However, Danielli, Rubinshstein and others assume that an aqueous solution is present on both sides of the membrane. In this case it is hard to understand the orientated distribution of lipoid molecules in the form of a double-layer palisade. It is also difficult to imagine selective ionic permeability across such a
204 system.

The theory proposed by Hertz (1947) is even more fantastic. This investigator assumed that on excitation, the orientated lipoid molecules of the membrane turn through 180 degrees as a result of which the charge of the cell surface becomes negative instead of positive. It is not clear what are the forces turning the molecules in one direction or another. Besides, this hypothesis does not explain the decrease in electrolytic resistance (impedance) of the cell after excitation.

The sorption theory gives a much simpler explanation without additional assumptions. The main reason for the appearance of both action currents and resting currents is the release of electrolytes bound to proteins, and the loss of phase properties of protoplasm. As a result, the rise in the potential of injury and the action potential will be oriented with the "minus" sign in the direction of the electrodes, and with the "plus" sign in the direction of the nonstimulated protoplasm (Figure 120). If, following excitation or damage by any method, all the electrolytes were completely

released, the rise in potential at (a) and at (b) (Figure 120) would be equal and mutually neutralizing, because the potential of injury would be equal to the action potential. However, from our point of view this is not at all necessary. On the contrary, the known facts indicate incomplete release of electrolytes, for example upon mechanical damage. In the chapter on permeability it has already been mentioned that myosin obtained from muscle fluids at low temperature is partially bound to potassium (Ernst and Schaffer, 1928; Ernst and Fricker, 1934a. 1934b; Reginster, 1937, 1938a, 1938b; Szent Györgyi, 1947, and others). Reginster extracted myosin from resting and from previously stimulated muscles directly or through the nerve. His data are given in Table 34.

Table 34

Ratio of bound to diffusible potassium in calf muscles of the frog: in the resting state and after excitation with electric current (according to Reginster, 1937; from Troshin, 1956)

Condition of muscles	Potassium ratio
Resting muscles	2.6;3.1;2.9
Twin muscles after direct excitation	1.8;1.4;1.6
Resting muscles	2.7;4.1;7.5
Twin muscles after excitation through the nerve	1.6;1.8;1.6

It is quite obvious that mechanical damage by no means releases all the potassium, and that this release is more complete after excitation (Table 34).

If this is so, then in Figure 120 the jump at b should be higher in its absolute value than at a, as a result of which the action potential will be
205 higher than the potential of damage, and this is what had to be proved. In addition, our theory explains both exit of potassium and increase of electroconductivity on excitation.

Chapter 3. Bioelectric Potentials and Cell Metabolism

Statement of the Problem

As a result of a difference in potential on the cell surface, electric current is formed. It is self-evident that this process may take place only at the expense of certain energy sources. In the living cell such a source may only be cell metabolism. This assumption, stated in a general form, follows from the law of conservation of energy, and does not necessitate any special physiological confirmation. Therefore, any theory of bioelectric potentials should eventually be a "metabolic" theory.

In this respect the task of the physiologist studying bioelectric processes is to find a practical solution to two problems. The first is to determine the direct cause for the appearance of difference in potentials. The second is to determine the mechanism which transforms the energy of cell metabolism from chemical to electric.

Strictly speaking, the membrane theory only answers the first of the two questions. This theory states that the reason for the difference in bioelectric potentials is a different concentration of intra- and extracellular electrolytes. According to this theory, bioelectric potentials are potentials of concentration of elements obtained in the presence of semipermeable membranes. The membrane theory gives no specific answer to the question of how these differences in concentrations arise in the cells, but it is understood that they can be formed only by means of various metabolic processes. In other words, the membrane theory says nothing definite on the possible transformation of chemical into electric energy.

Unfortunately, the data in the literature as yet give no satisfactory answer to this question.

Effect of Inhibition of Different Cellular Metabolic Processes on Bioelectric Potentials

Many studies of this kind attempt to prove a relationship between bioelectric potentials and metabolism, by inhibition or elimination of some aspect of metabolism. They record the study of the effect of this inhibition on the magnitude of these potentials. Thus Furusawa (1929) observed a decrease in the demarcation potential in crab nerve under anoxic conditions. Gerard (1930) showed that in the frog nerve the demarcation difference in potentials decreased during asphyxia. It was shown that oxygen lack in the damaged segment did not lower the difference in potential, while asphyxia of the normal part of the nerve resulted in a reversible decrease in this difference.

Later, Muzheev and Borzdyko (1933) caused a decrease in the resting
206 current of skeletal muscles, by poisoning them with sodium cyanide and monoiodoacetate. Their data were later confirmed by Michelson (1935). This investigator came to the conclusion that the reason for this decrease was a severe disturbance in tissue structure.

The studies of Karaev (1937), and a series of studies by Lebedinskii et al. (Lebedinskii, 1939; Mozzhukhin, 1948, 1950a, 1950b, 1953; Lebedinskii and Mozzhukhin, 1950, and others) were planned according to the same pattern. Lebedinskii and Mozzhukhin studied the effect of metabolic inhibitors on the extent and rate of decrease of injury currents and of excitation potentials in skeletal muscles.

At first it may seem that this method of investigation should lead to the solution of the problem as to what form of metabolism serves as a source of energy for the formation of potential differences in the cell. But if one analyzes the results obtained in this manner more carefully, it is not difficult to detect the basic shortcoming of this method of study.

All methods of metabolism inhibition to some extent decrease the value of potential difference. However, it cannot be concluded from this that all these methods eliminate the energy sources of biocurrents, since they may all cause specific damage to the cells for other reasons. They may lead to changes in dispersion of the cell colloids, to decrease in their hydration, to denaturation of proteins, and to splitting off of electrolytes bound to these proteins, etc. Changes of this kind may not be observed under the microscope in the initial phase. All this should most markedly

lower the difference in cell potentials (depolarize the normal surface of the cell). It is almost impossible to differentiate between this nonspecific damaging effect of the toxin-inhibitor and the direct effect of cell metabolism inhibition on biopotentials.

For example: frogs, insects, crustaceans and other animals were subjected to reversible asphyxia (Nasonov, 1930, 1932a, 1932b; Aleksandrov, 1932 and others). It was easy to detect microscopically that all the tissues were in a state of marked paranecrosis. Nuclear structures appeared in the cells, granule formation ceased, vital staining of protoplasm and nucleus was observed, etc. This condition was accompanied by the appearance of electronegativity of the normal surface, i.e., it should have caused a decrease in injury currents. On renewal of the action of oxygen, all these phenomena rapidly disappeared.

What is the cause of all these changes?

It might be concluded that this happened because of exhaustion of energy resources essential for the maintenance of normal cell structures. However, it is possible that direct poisoning by hypoxic metabolites, in the first place by lactic acid, took place.

The possibility of such nonspecific poisoning is even more probable after the action of poisons inhibiting metabolism. For example, some of them (sodium fluoride, monoiodoacetate and others) in doses used by biochemists for inhibition of metabolism, shorten the time of survival of frog muscles 3-20-fold (Ushakov, 1953a), causing such nonspecific signs of poisoning as muscular contractions (Ushakov and Dzhamusova, 1954).

207 By this we do not imply that there is no direct relationship between cell metabolism and biopotentials. On the contrary, as already mentioned, we think that such a connection is in principle so obvious that it does not even require confirmation. But we assume that the methods of study of these relationships chosen by Furusawa, Gerard, Lebedinskii and others does not allow convincing conclusions to be drawn as to the energy sources of biocurrents. Strictly speaking, these studies only show that poisoning by inhibitors may also cause negativity of the surface of the cell, as does treatment with a great number of other nonspecific poisons. These studies do not contribute anything new to the actual understanding of bioelectric processes.

Relationship between Bioelectric Potentials and Cholinergic Activity of Cells

The orthodox theory of transmission of excitation from one cell to another involves the excretion of acetylcholine by nerve endings. Recently, attempts were made to apply this mechanism to the appearance of excitation in nerve conductors and muscle fibers (Barnes and Beutner, 1941; Koshtoyants, 1944; Nachmansohn, 1946, and others). The reason for this was the discovery of acetylcholine and cholinesterase not only in nerve endings but also in certain nerve trunks as well as in the electric organs of fish. On the basis of model experiments, Barnes and Beutner (1941) tried to prove that acetyl choline itself can create a sufficiently high membrane potential. At the same time, Nachmansohn, 1940, postulated that acetylcholine excreted by the nerve fiber at the moment

of excitation acts in a depolarizing way on the internal surface of the cell membrane, increasing its impermeability. Due to this, electronegativity appears on the excitated surface. Thus, the theory of Nachmansohn should be considered only as a supplement to the membrane theory, insofar as, in his opinion, acetylcholine cannot be the cause of formation of bioelectric potentials being necessary only for their detection.

Attempts were made to confirm this theory by direct experiments. As is well known, eserine and prostigmine inhibit cholinesterase—(the enzyme splitting acetylcholine and arresting its action after termination of the stimulation). It was suggested that after the action of eserine on the nerve, acetylcholine appearing at the moment of stimulation will not be able to stop its depolarizing effect, because of the elimination of cholinesterase action. As a result of this a block should form which does not let the waves of excitation through. The experiment has shown that eserine at a very high concentration (1:250) rapidly annihilates action currents and excitability (Bullock, Grundfest, Nachmansohn, Rothenberg, 1947). However, prostigmine, which like eserine is an anticholinesterase, shows no such effect even at a concentration of as high as 4 mg per cent. Acetylcholine itself also does not block conduction by the nerve even at such unbelievably high concentrations as a 2% solution.

In order to explain all these data from the point of view of his theory, Nachmansohn had to make the following, quite arbitrary assumptions: 1) Acetylcholine does not permeate the cell membrane, but depolarizes
208 it only from the inner side; 2) eserine penetrates freely through the cell membrane; 3) prostigmine does not penetrate it at all. Only by accepting these assumptions could the results obtained be interpreted as confirmation of his theory.

These considerations have recently been completely disproved by Ginetsinskii, by very clever and simple experiments performed in cooperation with Barbashova (Ginetsinskii and Barbashova, 1949). These authors first of all showed that acetylcholine penetrates easily into muscle cells, and therefore showed Nachmansohn's first assumption to be wrong.

It has further been shown experimentally that the difference in effect of high concentrations of eserine and prostigmine on excitability is not at all associated with their different permeabilities through the cell membrane, since there is no difference in the action of eserine and prostigmine on cholinesterase activity of the intact muscle. Eserine completely inhibited cholinesterase in a dilution of 1:10,000, while prostigmine completely inhibited enzyme activity at a dilution of 1:2,000,000.

These experiments entirely disprove the second assumption of Nachmansohn, that prostigmine, in contrast to eserine, does not penetrate at all across the membrane into the cell.

Why then, does eserine nevertheless inhibit the excitability of muscle, while prostigmine does not?

In the first place the dose of this poison used by Nachmansohn was extremely high, 400 times greater than that necessary for complete inactivation of cholinesterase.

There are reasons to believe that the inhibition of excitability by eserine is caused by nonspecific damage to protoplasm due to the use of a very high concentration of this substance. In order to elucidate this question, Ginetsinskii and Barbashova used our method of vital staining. By staining the muscle with neutral red in the presence of prostigmine, it was

seen that none of the concentrations used increased the sorption of the poisoned muscle, as compared to the control. Table 35 shows experimental data obtained by these authors, with the highest two concentrations of the poison. The authors rightly conclude that prostigmine has no damaging effect on muscle, and therefore does not alter its excitability.

209 Different results are obtained on studying the effect of eserine (Table 36).

It follows from Table 36 that a concentration of 1:1,000 causes a considerable increase in the sorption properties of the poisoned muscle. Using 1:250 solution of eserine, which according to Nachmansohn is necessary for inhibition of conductive function, damage goes so far that the poisoned muscle sorbs 2.5 times more dye than does the control.

Thus, the work of Ginetsinskii and Barbashova not only disproves the "acetylcholine" theory of Nachmansohn, but also indicates the main source of its errors—the arbitrary endowment of the hypothetic cell membrane with the ability of letting through or preventing the passage of these or other substances. Thus the experimental testing of these properties is not beyond criticism.

Oxidation-Reduction Theories of Bioelectric Phenomena

In terms of both membrane and phase theories, the direct cause for the appearance of bioelectric potentials is the difference between the intra- and extra-cellular ionic concentrations. A different point of view is provided by the theory which explains the appearance of bioelectric potentials by the different levels of oxidative-reductive (redox) potentials inside and outside the cell, arising as a result of cellular metabolism. This was first postulated by Lund (1928). This author showed that the difference in electric potentials on an onion rootlet is directly proportional to the intensity of respiration; increase in the intensity of the latter being accompanied by an increase of the positive charge. Similar relationships were observed by the author in the skin of frogs.

The facts cited by Lund cannot themselves serve as proof of his ideas, since greater or lesser intensity of oxidative-reductive processes may lead to a difference in ionic concentrations. Another objection to Lund's theory (Rubinshtein, 1947) was that the redox potential can be directly transformed into a difference of electric potentials only in the presence of metallic electrodes, which are not present in the cell. This very serious difficulty for the oxidative-reductive theory was discussed by Korr (1939), who indicated that in certain cases a nonmetal, for example graphite, may possess electronic conductivity. On this basis, Korr quite arbitrarily ascribed to the cell membrane the ability to conduct a current of electrons, characteristic of metals.

In the USSR the oxidative-reductive theory is supported by Kometiani (1945), who, by the way, assumes that only part of the measured difference in potentials of the cell may be ascribed to redox potential, while the other part should be considered as a concentration potential. Kometiani has shown a model of two aqueous solutions differing in their redox potentials, and divided by a gelatine membrane treated with quinhydrone, giving a potential difference reaching 18 millivolt. It should be pointed out that in

Kometiani's model there is no certainty that the ionic concentration on both sides of the membrane is the same, and therefore the electromotive force obtained may also be considered as a concentration potential.

Table 35

Effect of prostigmine on sorption of the dye (0.25% neutral red) (according to Ginetsinskii and Barbashova, 1949)

Concentration of prostigmine	Amount of dye sorbed (mg per 100 g of tissue)		
	control	experimental	experimental (% of control)
1:250	2.08	1.80	90
	0.96	1.02	106
1:1000	1.08	1.12	104
	0.94	0.86	91

Table 36

Effect of eserine on sorption of the dye (0.25% neutral red) (according to Ginetsinskii, 1949)

Concentration of eserine	Amount of dye sorbed (mg per 100 g of tissue)		
	control	experimental	experimental (% of control)
1:250	1.18	3.00	254
1:1000	1.76	2.08	118
1:20000	2.96	3.44	116
1:100000	1.16	1.22	105

210 The strongest argument against this theory is the fact that in reality no considerable difference in redox potentials is observed inside and outside the cells. Numerous measurements of intracellular potentials given in the paper by Michaelis (1926) indicate that in the majority of cases the intracellular redox potential is the same as that of the surrounding medium This being so, no matter what the properties of the cell membrane are, no difference in potential could appear.

There is evidently no oxidation-reduction barrier in the cells. If muscle fibers previously stained with methylene blue are placed in an anaerobic medium, the dye whose reduced leukoform is colorless rapidly loses its color. On transfer of the muscles back to the air, methylene blue is oxidized again, turning blue literally within a few seconds. This means that within these few seconds the redox potentials inside and outside the cells became equal, since the transition point of oxidized methylene blue into its colorless leukoform is very close to water in equilibrium with air. After the onset of the redox equilibrium, the muscle fibers are nevertheless capable of forming characteristic action currents and resting currents. Such instantaneous equilibration of redox potentials of the cells and medium was observed by us in infusoria parasites in the stomach of ruminants (Ophryscollecidae), under conditions of very low pH. In the case of the infusoria

too, on exposure to air the intracellular potential became immediately equal to that of the abundantly aerated external medium (Nasonov, 1932b).

It may nevertheless be assumed that a certain almost undiscernible difference in intra- and extra-cellular pH does exist, due to continuous metabolism, maintaining a redox gradient. However, our experiments with muscles fixed in formalin somewhat contradict this possibility (Nasonov and Aleksandrov, 1944; Nasonov and Speranskaya, 1948; Golovina, 1948). Obviously, in this case metabolic processes cannot be the source of differences in redox potentials. Nevertheless, we have shown that injury potentials may be observed in these muscles many hours after fixation, reaching 20 millivolt. These currents in muscles completely devoid of metabolism may be caused by the local effect of cutting, high temperature, and electric current, the "minus" appearing always on the damaged surface.

This phenomenon may well be explained by our theory. Formalin is evidently capable of killing the muscle, but for some time preserves certain native properties of its protoplasm proteins, in particular its phase properties and its capacity to retain bound potassium. Thus, Tarusov (1941) has observed that muscles and nerves fixed in formalin retain the capacity of polarizing resistance to direct current for a long time, this capacity disappearing as a result of high temperature, or with action of alcohol. Kronfeld and Scheminsky (1926) observed that on fixation of fish egg cells with alcohol or acid fixatives, coagulation of protoplasm occurred, its salts leaving rapidly to the outside, due to the electroconductivity of the external solution. Formalin differs from other fixatives in that the egg cells do not become turbid in it in spite of gelatinization, and electrolytes do not leave
211 them. Injury of all kinds releases potassium from the formalinized protoplasm, as a result of which the difference in potentials is detected. Such an unstable potassium-protein complex could be formed in the cell only due to previous metabolism. This metabolism, however, is absent at the time of measuring the damage potential of the formalinized muscles and consequently excludes the possibility of an oxidative-reductive explanation of its nature.

Phase Theory of Bioelectric Potentials and Cellular Metabolism

According to the phase theory of bioelectric potentials, the immediate reason for the appearance of electromotive force on the surface of the cell is the difference in ionic concentrations in two regions of the protoplasm. This difference is not preexistent, but occurs suddenly after damage or excitation, due to the splitting of an unstable chemical compound, the protein-salt complex. The reason for this splitting should be considered a reversible denaturation of proteins in the initial stages.

While earlier Nasonov and Aleksandrov (1940) assumed the existence of such a "protein-salt complex", now, as a result of the data in the literature given in the chapter on cellular permeability, a greater degree of probability exists. We have also mentioned facts showing that denaturation of native proteins containing bound potassium enables the latter to pass into solution in the ionized form.

We consider that this exothermic reaction, splitting electrolytes from protein due to the action of an irritant or a damaging agent, is one of the

most important processes in metabolism, since it leads to the formation of potential differences between the excited and resting surfaces. This reaction is one of the links of intracellular metabolism, and it would be incorrect to define it as an expression of a certain "structural energy" as opposed to the energy of metabolism, as is done by some physiologists. The term "structural energy" was introduced into physiology by Warburg (1928) to designate energy used for the maintenance of the specific properties of semipermeable cell membranes. We are now forced to reject this term in the above sense, since we question the reality of the cell membranes themselves. However, if by these structures one is to understand the structure of protein or other molecules, then energy is evidently necessary for the maintenance of certain labile chemical compounds. The special term "structural energy" may only cause confusion. This became especially apparent after the studies of Engelgardt (1941), who discovered the enzymatic properties of myosin and showed that protoplasm proteins cannot be divided into structural and nonstructural components.

It is known that after excitation, the split-off electrolytes again combine with the proteins. It is quite obvious that this restorative process, which is most probably connected with renativation of proteins, requires energy expenditure. This may take place only at the expense of some metabolic processes, probably using high energy compounds as their energy source. But as yet we know little about these bonds, and their elucidation remains a task for the future.

Part IV

212

SPREADING EXCITATION AND ITS CONNECTION WITH THE LOCAL REACTION

Chapter 1. History of the Problem

Reversible changes in the organism observed at the site of protoplasmic stimulation were designated by us "the local reaction of the organism to external influences". According to the terminology of N. E. Vvedenskii, the sum total of these changes may be defined as a local, stable excitation, or parabiosis. The reversible changes observed in the living substrate we termed paranecrosis.

Under known conditions, the local excitation in nerve and muscle fibers is transformed into a spreading one, starting from the site of application of the irritant, and moving along the fiber with varying speed. This process is the basis of activity of the nervous system, since by this means a rapid connection and coordination of distant sections of the multicellular organism occurs.

A number of properties are common to local and spreading excitation. Among these are the electronegativity of the excited region, its refractivity, increase in permeability, decrease in dispersion of protoplasmic colloids, increase in vital staining, etc. However, these general properties still do not give a clear picture of the interrelationship between these two forms of excitation. It may therefore be assumed that a purely superficial similarity exists between the two.

In foreign, and to some extent in Soviet physiological literature this point of view predominates (Rosenblueth and Luco, 1950; Rosenblueth and Garcia Ramos, 1952). It is suggested that two qualitatively different excitations exist: local, and spreading. The former supposedly differs from the latter mainly in that it is quantitatively or, as they say, gradually, dependent on the intensity of the stimulus and does not obey the "all or none" law. The latter, on the other hand, does not depend on the strength of the stimulus and obeys the law.

The first physiologist to postulate the "all or none" law was Bowditch (1871), who observed that heart contractions cannot be increased or weakened by varying the strength of the stimulus. As long as the intensity of stimulation was lower than a certain threshold level there was no response ("none");
213 however, when intensity was even slightly above the threshold, maximal contraction occurred ("all"). Further strengthening of the stimulus did not affect the magnitude of response. This rule is observed especially easily in the heart, since heart muscle is a syncytium and represents one excitable unit. Nerve and skeletal muscle are more difficult to observe since they consist of a large number of excitable units possessing different sensitivities. Nevertheless, many studies on intact nerves, isolated fibers, and other tissues established beyond doubt the application of the "all or none" law to

conduction of impulses in nerve and muscle fibers of vertebrates and invertebrates; in filaments of the alga Nitella; in the stalk of the infusorium Vorticella, etc.*

Originally, the law was given an absolute meaning, assuming that protoplasm in no way reacted to subthreshold stimuli. However, Lucas (1905, 1909, 1911) observed that following such a stimulation of the nerve, a local reaction developed in the form of weak electrical potentials directly proportional to the strength of stimulation, being incapable of limitless spreading, and rapidly dying away. This local, subthreshold reaction, Lucas called the "local excitatory process". When the local process reached threshold value, a new phenomenon appeared explosively in this region—a spreading impulse ("propagated disturbance"), which basically differed from the former in that it obeyed the "all or none" law.

Lucas dealt in general with electric phenomena in the nerve, in the region of application of the stimulating electrode. He did not differentiate between the potential of the excitatory current and the potential arising in the nerve as a response reaction. This can be explained by the fact that at that time the technique of study did not permit the differentiation from each other of these two possible sources of subthreshold potentials. This task was successfully performed much later by two British physiologists, Katz (1937) and Hodgkin (1938). Their studies must be considered as a tremendous step forward in the investigation of the origins of the propagating impulse. Hodgkin used a very sensitive oscillographic method, attaching an electrode to a single crab nerve fiber placed in paraffin oil. He studied the relationship between the magnitude of electric excitation, and the magnitude of the electric response reaction on subthreshold stimulation of the fibers. By the use of a neat graphical method Hodgkin subtracted the value of the loop of excitatory current from the value of the observed electric response action. He thus obtained the value of the local electric action of the nerve in pure form.

Later, his data were confirmed by Pumphrey, Schmitt and Young (1940), using the giant fibers of Loligoforbesi, and by Hodgkin and Rushton (1946), using a single crab nerve fiber (Homarus vulgaris)**.

214 Essentially, this subthreshold local reaction completely corresponded to the concept of Vvedenskii (1901) of local excitation or cathodic parabiosis, with the difference that Vvedenskii studied parabiosis following prolonged action of the cathode in the case of which the response reaction was also more prolonged. British physiologists, however, observed this local reaction with very short-lasting electric stimuli, where the reaction itself was also very transitory. In this respect the discovery and studies of local electric responses were only a confirmation under somewhat different experimental conditions of Vvedenskii's theory; i.e., that the reaction observed in the immediate region of application of the irritant to living protoplasm is nothing but a manifestation of local excitation (parabiosis).

---

* Those interested in the history of the problem are referred to papers by Brücke (1930), Ritchie (1932), Adrian (1933) and Kato (1934).

** Lorente de No (1947) attempted to refute the existence of a local response to electric excitation of the nerve. However, the data given by him in support of this statement are not convincing. This was shown by Rosenblueth (1952) in his paper on the problem of subthreshold phenomena in nerves.

Following the studies of Katz and Hodgkin the term "all or none" is no longer properly applicable, since these authors observed a subthreshold local excitation.

It has further been shown that such reactions do not remain stationary but spread further with a decrement. This spreading capacity of a subthreshold local response was discovered independently of Hodgkin by the Russian physiologist Karaev (1938), using an entirely different method. Karaev, experimenting with sciatic nerves of frogs, used the phenomenon of summation of two stimuli following each other at a given time interval. The first stimulus was always applied at the same point, while the second one was applied at various distances from the former. Using this method with different variations, Karaev proved that the subthreshold impulse spreading with decrement may be observed at a distance of 1-2 cm from the site of stimulation.

Figure 121, taken from Hodgkin, shows that with an increased stimulus, the electric subthreshold response becomes increasingly stronger, and dies away increasingly further from the excitatory electrode. However, as soon as this stimulus even slightly exceeds the threshold value, instead of spreading with a decrement it begins to move with an increment (upper curve). Later, having passed a certain distance, the electric wave suddenly bends upward, and reaching the height of the normal peak running along the fiber, it moves along the nerve to its very end, with a constant strength, independent of the value of the initial stimulus.

Plotting the value of excitation on the abscissa and the value of the electric response reaction on the ordinate, a dependence graph may be constructed of the subthreshold reaction related to the magnitude of the stimulus causing it. As seen in Figure 122, plotted according to Hodgkin's data, this dependence is expressed by a smooth curve, convex in the direction of the abscissa.

Such curves describing the relationship of magnitude of the local reaction to the strength of the excitant were later obtained by Rosenblueth and Luco (1950) for impulses of direct current in spinal nerve roots of vertebrates (Figure 123 A), and by Rosenblueth and Garcia Ramos (1951), using the same tissue, with excitation by alternating current (Figure 123 B).

It follows from the above that the phenomena observed under the electrode after subthreshold stimulation are by no means as simple as it seemed at the beginning, and they require special theoretical explanations.
215 British physiologists tried to explain them in terms of the membrane theory of bioelectric phenomena (Rushton, 1937, etc.). They postulated that a subthreshold stimulus caused only a partial gradual depolarization in the cellular membrane, while with a threshold stimulus the irritant suddenly and completely depolarized the membrane. All the electric energy preexisting in the fiber was explosively released. These theoretical considerations differentiate in principle between the subthreshold local reactions and the propagation of excitation.

As opposed to this, the Soviet physiological school of Vvedenskii-Ukhtomskii developed a unitary concept of local and spreading excitation. Some representatives of this school, for example Makarov (1939), questioned the basis of the "all or none" law. These attempts were mainly concerned with the study of contractions of single muscle fibers, the myographic recording of which did not allow strict differentiation between local and spreading contraction.

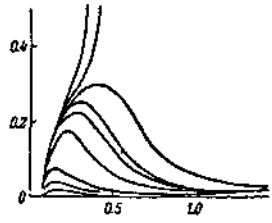


FIGURE 121. Development of local response with different intensities of the excitatory current (according to Hodgkin, 1938)

The abscissa shows time (in millisec); the ordinate shows voltage.

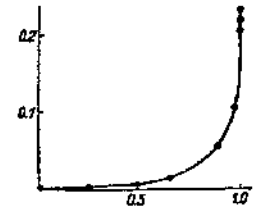


FIGURE 122. Relationship between local electric reaction of the crab nerve, and magnitude of excitation (according to Hodgkin, 1938)

The abscissa shows excitation (in fractions of the threshold); the ordinate shows the value of potential (in fractions of propagation).

Ukhtomskii (1926-1940) raised a number of substantial objections against the differentiation between local and the propagating excitation. He did not deny the facts of conduction of impulses according to the "all or none" law, but objected to the thesis that a propagating impulse of constant magnitude, independent of stimulus intensity, is an expression of "all" the energetic resources of the fiber. He rightly indicated that the possible "maximal excitation" of the cell should exceed many times the energy level of the propagating electric wave of excitation; he also pointed out that the constancy of the magnitude of this wave depended on mechanisms which automatically regulate its level, and which by no means reached maximal values (Ukhtomskii, 1940, p.238). Incidentally, we know of no statements made by Ukhtomskii on possible mechanisms present in protoplasm which regulate the magnitude of propagating excitation. Like Ukhtomskii, we also cannot agree with the many attempts to consider excitation as an explosive
216 process. We have many times observed the action of various stimuli on living cells and have never observed anything similar to "explosive reaction". Paranecrotic changes always occur gradually, and likewise, disappear gradually. The same may also be said of different physiological indexes, which always change gradually with the development of a stable local excitation (parabiosis).

These contradictions between local alterations which are always gradual, and sudden explosive manifestations of the propagating impulse, are by no means as irreconciliable as may seem at first sight. On the contrary, a more thorough analysis of the data has shown that conclusions from the "all or none" law necessarily follow the assumption that after an excitation wave, the nerve fiber reacts gradually at each of its points, exactly as in the region of application of the electric excitant. The following chapters are devoted to the foundation of this theory of "gradual" propagating excitation.

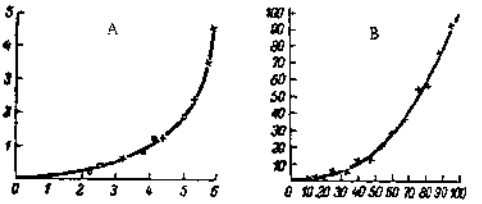


FIGURE 123. Relationship between the magnitude of the local reaction and strength of excitation

A—local cathodic response on excitation by pulses of direct current (according to Rosenblueth and Luco, 1950); B—local response on excitation by alternating current (150 cycles per second) (according to Rosenblueth and Garcia Ramos (1951).

The abscissa shows intensity of excitation (in arbitrary units); the ordinate shows the peak amplitude of the response (in arbitrary units).

Chapter 2. The Theory of Gradual Spreading Excitation

This now well-recognized theory is based on two well-founded hypotheses. These are as follows:

1) Transmission of excitation along the fibers is an electric phenomenon, i.e., takes place by means of "small currents", as suggested by Hermann (1885).

2) The local response reaction of protoplasm to excitation is always gradual, and never obeys the "all or none" law.

The Electric Mechanism of Transmission of Excitation

The suggestion that biocurrents participate in the conduction of excitation was made by DuBois-Reymond (1877). However, the electric theory of transmission was elaborated by Hermann (1885, 1889), and since later physiologists only developed and enriched it by new facts, we shall
217 give here the basic principles of his theory.

According to Hermann, a stimulus causes changes in the excited region, as a result of which this region becomes electronegative with respect to the adjacent sectors (Figure 124). The resultant difference in

potential causes the formation of extraordinarily intense local currents, due to low resistance in the region adjacent to the excited sector. These currents are directed from the resting to the excited sectors. Thus, the latter remain inactive, since they are positively polarized while the former are negatively charged and thus undergo excitation.

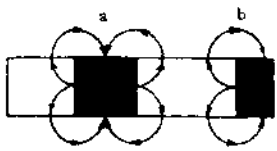


FIGURE 124. Schematic illustration of "small currents" on (a) stimulated and (b) damaged sectors of the fiber (according to Hermann, 1885)

"The consideration now stated", says Hermann in his concluding remarks (1885, p. 378), "is not hypothetical, but contains elements which in all probability will lead us, sooner or later, to the theory of the spread of excitation and to the understanding of the real meaning of electrical phenomena in animals. This is because sudden formation of negative polarization in close proximity to the originally excited site should stimulate the latter, while positive polarization might, in its turn, lead to return of the stimulated site back to the resting condition; ..... from the recently indicated elements one might construct a strictly mathematical theory of the propagation of the excitatory wave". Thus, according to Hermann, the process of conduction of excitation is considered as a propagated wave of electronegativity.

Hermann's views were fully confirmed by later investigators. Lillie (1936) and others built models on the basis of this theory, which reproduced Hermann's principle of electric conduction of impulses.

Figure 125 from the paper by Stämpfli (1952) illustrates schematically the contemporary theories of electric transmission of nerve impulse in a medullated fiber containing nodes of Ranvier.

The main experimental proof of the electric theory of excitation conduction may be classified under three headings: 1) experiments with "jumping" of excitation across a narcotized sector of small dimensions; 2) experiments in which excitation may "jump" from one nerve fiber to another; 3) experiments showing a connection between electroconductance of the
218 external medium and velocity of conduction of the impulse.

First Group of Confirmations. From Hermann's observations it follows that although the greatest current intensity is observed adjacent to the stimulated sector, nevertheless the loops of this current should encompass quite a considerable sector of the nerve. The intensity of the current loops running ahead may be sufficient to cause excitation in the nerve sector at a certain distance (but not beyond a certain limit) from the site of stimulation.

The possibility of such a jump of impulse through a sufficiently small sector of the block was demonstrated beyond any doubt by the studies of Verigo (1899), Blair and Erlanger (1936b), Hodgkin (1937), and Tasaki (1939). The last-named author performed very delicate experiments with a single nerve fiber. He eliminated one, two, and even three internodular segments and observed the conduction of the impulse. Even a greater number of segments can be eliminated and still give conduction of the impulse if its transmission is facilitated by making a salt bridge, connecting the nodes of Ranvier before and after the block. Prior to Tasaki's work, Osterhout and Hill (1930) used such salt bridges in experiments with the alga Nitella,

facilitating the transmission of negative oscillations across a blocked segment.

Second Group of Confirmations: If an electric loop of excitation can jump a small cross-block, it may be expected that under certain conditions it will also jump to a neighboring nerve fiber. Usually this does not happen, since obviously the insulating properties of the fiber sheaths promote the law of "insulated conduction of impulse". However, under certain experimental conditions this law may be broken, and excitation jumps to neighboring fibers (Kvasov and Naumenko, 1936; Katz and Schmitt, 1940). Finally, the well-known experiment of Galvani, in which stimulation jumps from a contracting calf muscle of a frog to a nerve of another nerve-muscle preparation placed across it, confirms the electric transmission of the impulse.

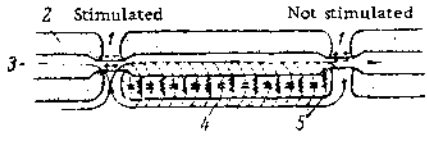


FIGURE 125. Basic scheme illustrating the "jumping" of excitation in a medullated nerve fiber (according to Stämpfli, 1952)

1—nodes of Ranvier; 2—myelin sheath; 3—axon; 4—current along nerve; 5—loss of current through myelin sheath.

Third Group of Confirmations: The theory of electric transmission of impulses is supported by experiments showing a connection between electroconduction of the external medium and the speed of conduction of the impulse. Insofar as, according to the theory of Hermann, the current of action closes through the external medium, any change in the electroconduction of the latter should change the speed of conduction of excitation. Indeed, investigations performed with different tissues and by various methods have shown that a decrease in the resistance of the medium leads to acceleration of conduction of the excitation wave. Thus, Auger (1933, 1936) demonstrated an accelerated conduction by filaments of the alga Nitella, by covering them with moist filter paper. Pond (1921) studied the velocity of conduction of an impulse in muscles of Limulus and in the sartorius muscle of the frog. Using a system of mirrors which directed the rays onto photographic paper, he measured the time between two waves of contractions. Using different ratios between Ringer's solution and isotonic sugar solution, Pond changed the electroconductivity of the external medium. The velocity of propagation of the wave of contraction was found to be directly proportional to the electroconductivity of the external medium. Pond thought that his balanced solutions differed from each other only in electroconductivity (he
219 took special care to maintain the same pH), but in relation to muscles, media with a different salt and sugar content are not equivalent.

Criticizing Pond's studies and others similar to it, Hodgkin (1939) entirely eliminated these shortcomings. His faultless and methodical data will be presented here in somewhat greater detail. He compared the speeds of conduction of excitation in the nerve by recording the action current in media with different electroconductivities, which were known not to damage the nerve. Thus, a single crab nerve fiber was initially immersed in sea water and transferred subsequently to liquid paraffin. Since by so doing the fiber remained surrounded by a small layer of sea water, changes in the ionic and chemical composition of the medium did not occur, and there

were only changes in electroconductivity. Transfer of the nerve fiber from water to oil and back into water lasted only a few seconds. As a result of the transfer from oil into sea water, the velocity of conduction of excitation in the nerve fiber increased by 14-40%.

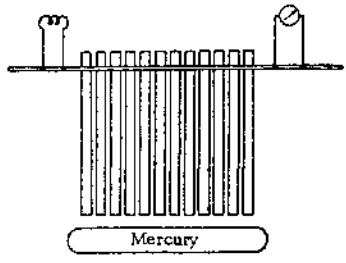


FIGURE 126. Apparatus for short-circuiting action currents in the giant axon of crab (according to Hodgkin, 1939)

Similar experiments were performed by Hodgkin on the giant axon of Loligo. The axon was immersed in a vessel with sea water and subsequently removed and kept in moist air. Here, too, media differing only in electroconductivity were compared. The percentage increase in speed of conduction on removal of the fiber from moist air into sea water was approximately 40%.

The following experiment of Hodgkin is singularly exact. A giant axon of a crab was placed on a number of silver wires, the bent ends of which could be immersed in mercury (Figure 126) or remain exposed. When the wire tips were immersed, electric current was passed through them, thus creating conditions for short-circuiting the action currents. By doing so, the speed of excitation conduction increased by 14-20%, while the medium in which the axon was kept did not change at all.

Recently, Huxley and Stämpfli (1949a, 1949b) created a conduction block in a single medullated nerve fiber, at the point where this fiber was surrounded by air. The block was removed by closing the action current through a liquid bridge.

The above observations and experiments, especially those of Hodgkin, were quite faultless in their methodology. They confirm beyond doubt the correctness of the electric theory of conduction*: Our further theoretical considerations will apply this theory as a working basis.

220 Basic Principles of Theory of Gradual Excitation

There are two basic hypotheses in this case. The first is the electric mechanism of propagation of excitation waves in the fibers. As pointed out in the previous chapter, this may be considered as a fully established fact. The second is that the local response to electrical excitation at each point of the nerve and muscle fibers is always gradual. This means that with increased intensity of excitation, the local electrical reaction to the excitation also increases gradually without jumps.

For the subthreshold region this assumption may now be considered proved by the thorough investigations of Hodgkin (1938). From the figure drawn according to his data (Figure 122) it will be seen that the uppermost

---

* It has also been shown that not only the excitation wave, but also damage spreads along muscle fibers by an electric mechanism. In the first case the mechanism is through the action currents, while in the second, it is through resting currents (Nasonov and Rozental', 1947).

point on the curve is the last subthreshold response. A slightly stronger excitation will already cause a propagating impulse.

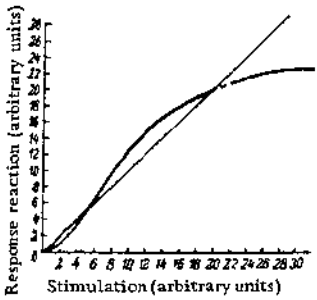


FIGURE 127. Relationship between the magnitude of response reaction, and intensity of stimulation

Our assumption is based on the fact that above this point the local reaction is also gradual (further proof will be given for this statement). If this is so, then further increase of excitation should lead to an increase in response reaction. However, this increase cannot be limitless. Sooner or later the fiber will respond by the maximum electrical reaction of which it is capable. In this region the curve will cease to proceed in this form, tending to become a straight line parallel to the abscissa. Consequently, the curve should acquire an S-shaped form (Figure 127). Such a curve is extremely typical. The vast majority of gradual relationships between a stimulus and any property of a living organism is usually expressed by an S-shaped curve.

An example (from Asmussen, 1934) is the curve of the relationship between the magnitude of local contraction and voltage of excitatory currents
221 in a single muscle fibers of frogs (Figure 128). This curve is comparable to that obtained by Hodgkin with nerve preparations (Figure 122). The only difference is that here the response reaction is measured not by the magnitude of electric response, as in the nerve, but by the amplitude of local contractions of the muscle fiber*.

An S-shaped curve of the electric response to excitation was also obtained with a single nerve fiber of Loligo which had lost its conducting ability (Schmitt and Schmitt, 1940). Subsequent discussions will be based on the electric theory of propagation of impulses described in the previous chapter. According to this theory, in each excited segment of the conducting fiber, when it becomes negative, originates electric current of sufficient magnitude to stimulate the adjacent sector (Figure 124). The latter, when excited, becomes in its turn electronegative in respect to the one following it, due to which current again arises, and so on. As a result of this chain of events the excitation wave is propagated along the fiber. A restoration wave immediately follows the excitation wave.

---

* What determines the S-shaped form of the curve of sensitivity of the conducting fiber? One answer is found in the interesting papers by Segal (1953/1954a, 1953/1954b, 1956). This investigator developed a theory according to which the reason for the appearance of excitation in the nerve fiber is a decrease in pH at the cathode, causing alteration of the protoplasm proteins initiating the excitation process. Segal has shown in a model experiment that the relationship between degree of alteration of native protein and the pH value is expressed by an S-shaped curve. He compared this curve with the curve of excitability of the fiber taken from our work (Nasonov, 1948b). Thus, according to Segal, the S-shaped nature of excitability of the nerve fiber may be considered as a reflection of the nature of sensitivity of protein molecules to the action of the stimulus. It seems to us that this thought merits attention.

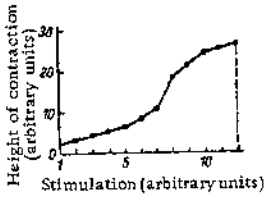


FIGURE 128. Relationship between magnitude of local contraction of single muscle fiber, and voltage of excitatory current (according to Asmussen, 1934)

If, as suggested, excitation at all points of the fiber is always in a gradual relationship to the intensity of stimulus, then it is obvious that the fate of the propagating impulse will depend on the ratio between the magnitude of current resulting in excitation, and that of the current arising as a response reaction. If, for example, at any point of the fiber the current formed will always be less than the current originating it, then propagation with decrement will take place, while at an inverse ratio the propagation will be with an increment. When the excitatory current equals the response current, spreading without decrement will be observed. We do not know beforehand what these ratios will be, therefore all possible cases will be analyzed.

The possible relationships between stimulatory current and effect current is set out in graphic form (Figure 129). The abscissa gives the voltage of excitatory current (x), the ordinate gives the voltage of current created by the former (y). Note that the bisectrix of the coordinate angle is characteristic in that the coordinates of any one of its points will be equal ($x = y$). For any point lying between the bisectrix and the abscissa, $x > y$, and for any point between the bisectrix and the ordinate, $x < y$.

As already mentioned, the ratio between the stimulatory current and the response current reaction should be expressed by an S-shaped curve. It is, however, impossible to say beforehand what position on the graph this curve will occupy. The first possible case will be discussed where the excitability of the nerve is characterized by a curve all the points of which lie below the bisectrix (Figure 129, curve α). In terms of the electric theory of transmission of excitation, it is not difficult to understand that in such a fiber any electric stimulus will produce an electric current of
222 lower voltage in the adjacent excitated sector. This latter current, in its turn, will cause an even weaker current in the neighboring sector, and so on. This is explained by the fact that the response reaction (y) here is always smaller than the stimulus causing it (x), since below the bisectrix $x > y$. As a result a decreasing spread is always obtained, as occurs, for instance, in the nerve fiber of Anodonta and certain other invertebrates.

In the second example described in Figure 129 by curve β, the curve differs from curve α in that all its points in the coordinate system are located higher than the corresponding points in curve α. In addition, curve β starts below the bisectrix, crossing it at a certain point b. It is easy to understand that in a fiber characterized by such a curve, all the stimuli which do not exceed the value oa will give a decreasing local reaction, since all the points of this segment of the curve are located below the bisectrix, and consequently the response reaction will always be lower than the stimulus causing it.

If, however, the value of the stimulus even slightly exceeds the threshold value oa, the course of events will sharply change on entering a region located above the bisectrix, where x is always less than y. Here the electric stimulus will give a stronger current, and the adjacent sector will form a

still stronger current, leading in turn to a rapid increase in potential in the course of its passage.

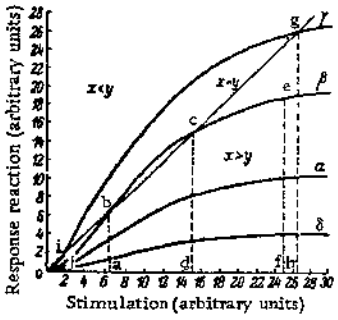


FIGURE 129. Excitability characterizing three main types of conduction: by fibers— α (spreading with decrement), β (spreading without decrement) and γ (automatic activity) (according to Nasonov and Rozental', 1952). For explanation see text.

On approaching the limit value of the response reaction curve β should again cross the bisectrix at point c. Let us consider the analysis of this extremely interesting and important point. At point c, located on the bisectrix, the magnitude of the effect equals that of the stimulus (x = y) and therefore, starting from this moment, each segment of the fiber, upon excitation, will generate a current of exactly the same magnitude as that which brought it to the state of excitation. As a result, the increase will cease and the potential of action ("peak") will be transmitted at the same magnitude without a decrement, to the end of the fiber. In that case, segment cd on the ordinate will determine the peak value.

It can be shown that the constancy of the value of this peak will be automatically maintained by autoregulation. Indeed, let us imagine that the value of the potential peak somewhat exceeded the value corresponding to point c, passing to the other side of the bisectrix. As soon as this happened, that region of the graph is reached where y is always less than x (i.e., the region of the decrement) and the magnitude of the propagated impulse, while falling, will begin to return to point c. How-
223 ever, while decreasing, the propagating potential will also not be able to jump to the left of the bisectrix beyond that point, since here it will be in the region of the increment (y > x) and will immediately begin to increase until reaching again the value of cd. Thus, reaching the point of intersection between the curve and the bisectrix, the propagating potential should acquire stability which will be automatically maintained during the entire time of its propagation by a specific autoregulation. It is obvious that no other special physicochemical mechanisms need be sought to explain this autoregulation.

Thus, from our theoretical consideration it follows that if an above-threshold stimulus is of smaller value than that of the peak, it should spread with increment until reaching the automatically regulated value of the constant peak (cd). It is interesting that this propagation increment was indeed found by Hodgkin on a fiber conducting without decrement, but was explained by the author in an entirely different manner*.

---

* Hodgkin assumed that in order for the subthreshold reaction to pass into a propagating impulse, not only the magnitude of voltage but also that of the strength of the action current has to reach threshold dimensions. According to Hodgkin, to achieve this condition it is necessary for excitation to encompass a segment of the fiber of a certain given length.

Let us now discuss the third possible case described by curve γ on Figure 120. This curve differs from the previous ones in that all its points in the coordinate system are located above the corresponding points on curve β. This curve also crosses the bisectrix at two points, but the first intersection takes place somewhere close to point o (i) and corresponds to a very low threshold of stimulation. The second intersection (g) corresponds to a high value of the regulated peak (h).

The threshold of excitability of a fiber characterized by such a curve γ is so low that very small stimuli, always present under natural or experimental conditions, will be above-threshold ones. Therefore the very weak local reaction caused by them will invariably increase in a snowball manner to a certain value, spreading along the entire length of the fiber. The fiber of the γ type differs from that of the β type in that its threshold is lower than the level of the ever-present excitations, and therefore such a fiber will always be "under their bombardment" while fiber β is as if protected from their excitatory effect by the high threshold. Thus, in the case of a fiber of the γ type we have a system in a special condition of instability, since with the slightest changes in the external environment the potential will rapidly increase to a constant value gh.

What will be the fate of such a fiber which spontaneously enters a state of excitation?

As is well known, such a fiber becomes refractory, i.e., incapable of responding by a propagating reaction to electric stimuli of any magnitude. From this moment it as if escapes the effect of weak stimuli, entering into reversible states, and on restoration of its previous condition may again respond to stimulation.

As can be easily understood from the graph, the curve of refractory (nonexcitable or weakly excitable) fiber will correspond to a curve approaching the abscissa with all its points (curve δ). On restoration of excitability of the fiber, the curve gradually acquires a position at the beginning of α, then of β and finally reaches the initial position γ in which, as has been
224 shown, the curve intersects with the bisectrix somewhere close to point o. Consequently, the fiber possesses a very low threshold. As soon as this happens, the fiber will again be in an unstable condition, and any very small impulse will cause a small local excitation. When this impulse propagates along the fiber, it will invariably grow to maximal dimensions and the fiber will again enter a refractory state. After that, recovery will again take place, and so on. Thus, a fiber having a gradual excitability of the γ type should be in a state of automatic rhythmic activity.

It is therefore clear that the frequency of excitatory outbursts, or the rhythm of automatic activity, should be entirely determined by the duration of a complete cycle of excitation, including the duration of its increase and recovery of the initial condition of the nerve. In other words, the rhythm of automatic activity depends entirely on that property of the fiber which Vvedenskii called lability. This theoretical conclusion was experimentally confirmed by us (Nasonov, Averbakh and Komarova, 1950; Nasonov and Averbakh, 1951). We shall return to this problem when automatic activity will be discussed in greater detail.

It need not be assumed that the automatically functioning fiber has a γ excitability along its entire length. It is sufficient to assume that only a small segment possesses such properties, while the remaining part is excitable according to the β curve type. In that case the whole system will

enter a condition of rhythmic activity, since the rhythmic spontaneous impulses originating in segment γ will be further transmitted to segments possessing β properties. Possibly the automatic cardiac regulatory centers, and other automatically functioning organs (undulating cilia, flagella, etc.), possess γ excitability.

From the above data it follows that the three basic types of nervous and muscular activity (local decreasing excitation of the α curve type; excitation spreading without decrement corresponding to curve β ; automatic activity corresponding to curve γ) should be transformed from one into the other upon simple increase or decrease in excitability of the fiber. This has been experimentally confirmed. Indeed it is known that lowering the excitability of the automatic center of the heart by narcotics can arrest its rhythmic activity, but the capacity to respond to different stimuli by a single contraction is preserved (transition from γ to β). On the other hand, increasing excitability by the action of dehydrating agents, or by agents precipitating calcium, can transform any muscle or nerve into an automatically functioning tissue (transition of β into γ). This assumption is confirmed by analysis of numerous data in the literature and by our own experimental data (Nasonov, Averbakh and Komarova, 1950; Nasonov and Averbakh, 1951; Nasonov and Suzdal'skaya, 1954).

These are, in general terms, the main postulates of the theory of "gradual" propagation of excitation. A number of known facts associated with the origin of the impulse and its propagation are not only well explained by this theory, but included in it. These comprise: 1) the gradual development of subthreshold potentials; 2) their propagation with decrement; 3) a distinctly demarcated threshold dividing the local effect from the pro-
225 pagating impulse; 4) increment of the above-threshold impulse to constant values, and propagation of the impulse during this increment; 5) automatic regulation of the magnitude of the propagating impulse during its passage; 6) transition from local reaction to propagating impulse without decrement, and further, to automatic activity following successive increase in excitability, and so on.

However, this coincidence between the theory developed here and the facts already known may seem insufficiently conclusive. Therefore, we performed a series of original studies aimed at testing this data. Discussion of these studies now follows.

Supramaximal Potentials

According to the theory developed by us, excitation exceeding somewhat the threshold value oa (Figure 129) should cause (in a β type fiber) an increasing, and at the same time steady, action potential. It has already been mentioned that this increase cannot be infinite, and that the action potential reaching point c will move along the fiber, maintaining a constant value equalling dc, since at point c this value acquires the ability to be regulated automatically.

What will be the case, however, if the value of the initial stimulus will be considerably higher not only than the threshold value, but also than the value of the constant propagation peak?

Let us assume that the value of this initial excitation, which we shall further call supramaximal excitation, equals 'of' (Figure 129). Then the value of the local response reaction will be expressed by the segment 'ef'. This value should be greater than the constant peak 'dc' and less than the stimulus 'of' which caused it (this potential we shall further call the supramaximal potential). Point 'e' is located to the right of the bisectrix in the region of decrement. Consequently, spreading along the fiber, the potential 'ef' will drop until the value 'dc' is reached. From here, due to the reasons given above, it will move along further, maintaining its constant value which will be automatically maintained to the very end of the fiber.

It has been stated above that if the action potential is even only slightly higher than the value of 'ab', it should spread with an increment until it reaches the constant value of the peak 'dc' at point 'c'. This propagation with increment was already noted by Hodgkin (1938). Now we have been convinced that the supramaximal potential 'ef' should spread with a decrement until it reaches the same stable value of the constant peak 'dc' at this very point 'c'. This assumption, which is a corollary of our theory, requires experimental confirmation.

It is first necessary to determine the value of the supramaximal potential caused by a supramaximal excitation. One should then compare the value of this potential with that of the constant propagation peak and the length of the fiber segment along which the gradually decreasing propagating impulse reaches a constant peak value should be determined.

Now follows the description of the experiments (Averbakh and Nasonov, 1950). These are given at some length, because we consider them one of
226 the main confirmations of these theoretical considerations.

In all the experiments sciatic nerves of frogs, isolated from the spinal cord to the hallux, were used. During the experiments, the stimulating electrodes were always placed on the nerve in the direction of the spinal cord and the recording electrodes on the opposite, thinner part of the nerve. Thus the fibers under the recording electrodes always conducted impulses at sufficiently strong excitations.

A cathodic oscillograph was used to record the excitation potentials.

As is well known, the oscillogram of the propagating impulse in a multifibered nerve should be evaluated, not by the apex of the peak, but by its area (Erlanger, Bishop and Gasser, 1926). It is not difficult to study the propagating peak if it is sufficiently removed from the site of electric excitation. Also the determination of the value of these areas on the oscillogram by means of a planimeter is not difficult. The loop of the excitatory current can be reduced to a minimum or eliminated under these conditions by a grounded silver plate (tapping). It is much more difficult to determine the value of the local or propagating impulse at the site of excitation or in its immediate vicinity, when the image of the excitatory potential fuses with that of the action potential. However, even in this case it is possible to differentiate quite accurately between these two values.

This was achieved using the experimental scheme described in Figure 130. Here a and b were recording electrodes (O—oscillograph). The tapping of the current was monophasic since the electrode a led the current away from the killed end of the nerve (shaded segment). The distance between a and b was 9-12 mm. Electrode b was grounded, serving at the same

time as one of the stimulating electrodes. The other stimulating electrode c was at a distance of 45-47 mm from the former. The value of the local reaction under the electrode b was determined. For this purpose the nerve was first stimulated by a series of electric shocks in such a way that the cathode corresponded to c and the anode to b. The impulses arose only at c, reaching electrode b after a certain time interval, following the recording of the loop of stimulating current at b by the oscillogram.

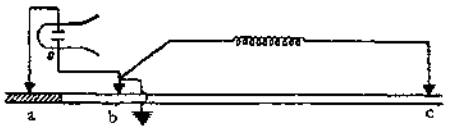


FIGURE 130. Apparatus for determination of local reaction (according to Averbakh and Nasonov, 1950). For explanation see text.

227

Such an oscillogram is shown in Figures 131, D and 132 A. The edge directed downward is the positive loop of the current at b*. At a certain distance from it there was a hump. This was the peak which reached b from a long distance after a certain time interval, reaching a constant value and having an opposite (negative) sign. After taking such an oscillogram, we changed the direction of the stimulating current. Then the impulses arose at b as the cathode and the images of the local potential of stimulation and of the negative loop of the stimulating current fused on the oscillogram (Figure 131 C and 132 B). In order to find the area corresponding only to the potential of the local excitation, using these two oscillograms, it was necessary to subtract from the total area (Figure 131 C and 132 B), the smaller area corresponding to the loop of the stimulating current. This area is shown in Figure 131 D and 132 A in the form of a sharp downward directed peak. This subtraction is recorded graphically by superimposing Figure 132 A on Figure 132 B. A difference in the form of the shaded part of Figure 132 C is then obtained. The area of this difference is determined on the Figure by a planimeter and subsequently compared with the area of the established propagation peak (Figure 132 D), obtained by drawing the contour of the propagation peak on Figure 132 A or 131 D. However, such a comparison is admissible only if it is certain that in both positions of the poles on excitation, all the nerve fibers, without exception, are functioning. In such a case the strength of stimulation should exceed the threshold values for all fibers comprising the nerve.

228

To prove the case, it is necessary to increase gradually the strength of stimulation, starting from its subthreshold value. At first only the loop of current on the oscillogram is seen, directed downward, and no hump corresponding to the peak is observed. On reaching the threshold of the most sensitive fibers a small hump suddenly appears (Figure 131 B) corresponding to the propagation peaks of these fibers. With further increase in the stimulus, the area of the peak on the oscillogram correspondingly increases. This depends on the fact that less and less sensitive fibers join the reaction consecutively. Finally, such a strength of stimulation is reached where its further increase does not cause an increase in the area of the peak. The dimensions of the latter become constant and do not depend on the strength of the stimulating current.

---

* The amplitude of this loop was directly proportional to the voltage of stimulating current, which made possible a precise determination of the relative strength of excitation in one series of experiments.

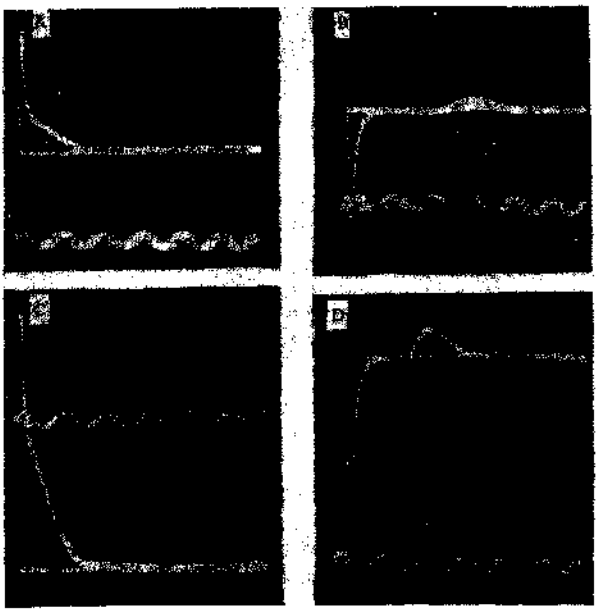


FIGURE 131. Oscillograms obtained by the use of the apparatus illustrated in Figure 130 (according to Averbakh and Nasonov, 1950)

A, B—stimuli of threshold strength; C, D—stimuli upon which a constant value of the peak is established; A, C—cathode of electrode b; B, D—cathode of electrode c. Time scale: 1 period corresponds to 1 millisecond.

From this point all the fibers in the nerve conduct the impulse and begin to function as a unit. All our measurements were made under such conditions and there was, therefore, no necessity to experiment with single fibers, as was performed by Hodgkin, who studied subthreshold potentials.

The determination of the local potential following supramaximal excitations was made in the following manner:

1) A prepared nerve was placed on electrodes with the proximal end to c (see Figure 130).

2) The end of the nerve at a was killed by heating.

3) Excitation was performed by separate electric shocks (100 per sec) with the minus sign originally at c. Subsequently, the excitation was gradually strengthened from subthreshold values until the first traces of the propagating peaks appeared on the oscillograph screen (Figure 131 B). This picture was photographed and the amplitude of the loop of current measured.

4) The stimulus was increased to a constant value of the peak (Figure 131 D). This picture was photographed, after which the poles were changed and the corresponding picture on the screen (Figure 131 C) was photographed again.

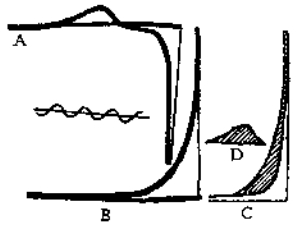


FIGURE 132. Graphic subtraction of areas, for the determination of the value of local reaction (according to Averbakh
229 and Nasonov, 1950)

A, B—oscillograms obtained by the use of the apparatus described in Figure 130. A—recorded at the position c of the cathode (the edge directed downward is the loop of current, the hump above is the peak arriving from electrode c; B—taken at position b of the cathode (the edge directed upward is the local reaction plus the loop of current); C—result of superimposition of A on B, the shaded area corresponding to the value of the local potential. D—area of the peak taken from A, for comparison with the shaded area of the local potential C.

5) Subtraction of the area was performed on the graphic representation as described above. The difference in areas was measured by a planimeter, corresponding to the value of the local potential at point b (Figure 130). Later, the area of the peak running from point c (Figure 130) was determined on the picture, and the value of the propagating peak (Table 37) recorded. The amplitude of the loop of current was measured (on Figure 131 D). The ratio between this value and the amplitude of the loop of current at a threshold stimulation was a measure of the strength of stimulation in threshold units (Table 37).

6) The stimulus was increased to a maximum, after which all the procedures as described in paragraphs 4 and 5 were repeated.

Table 37 shows the results of measurements performed in this manner (total of 21 measurements).

On the basis of the given data the following conclusions may be drawn. Firstly, on stimulation exceeding the threshold 2-4 times, the value of the local reaction is 1.5-2 times higher than that of the spreading peak. Further, the local reaction to supramaximal stimulation is gradual and does not obey the "all or none" law. At an average strength of stimulation of 1.8 thresholds this reaction is approximately 142%, while at 4.2 thresholds it reaches 193%.

If the local potentials may indeed exceed the value of the propagating peak 1.5-2 times, then it is obvious that on spreading, this potential should gradually decrease until it reaches a constant value. In other words, in a certain sector it should move with decrement. In this connection we decided to determine the duration of this decremental spreading.

230 This was accomplished by the use of an oscillographic recording of the spreading potential, at various distances from its place of origin. The main experimental difficulty was the necessity to reduce to a minimum, or completely eliminate, the stimulating current in the tapping of the loops. In order to achieve these results two methods were employed. Firstly, in all the experiments with stimulation, electrodes were used in which the anode was a grounded U-shaped silver plate and between them a wire was placed, serving as the cathode (Figure 133, A, c, d, e and Figure 133, B, f, g). The U-shaped grounded anode also served as the plate tapping the loop of

current. In addition, in some of the experiments (where possible) an additional auxiliary grounded silver plate was used (Figure 133, B, e)*.

Table 37

Local potential following supramaximal stimulation (according to Averbakh and Nasonov, 1950)

No. of experiment	1st determination—local reaction upon establishment of a constant running peak		2nd determination—local reaction upon maximal permissible excitation	
	magnitude of stimulus (arbitrary threshold units	value of local potential (% of constant peak)	magnitude of stimulus (arbitrary threshold units	value of local potential (% of constant peak)
1	1.4	133	2.1	180
2	1.6	169	2.1	193
3	3.5	133	5.0	168
4	1.5	100	5.0	166
5	2.0	268	2.8	342
6	1.9	108	7.0	203
7	2.1	283	2.5	303
8	1.7	133	7.3	221
9	1.7	135	2.6	182
10	1.5	139	2.1	180
11	1.6	97	5.0	179
12	—	133	—	—
13	1.5	126	2.6	166
14	1.5	103	5.0	167
15	1.7	119	7.2	232
16	—	144	—	—
17	1.5	119	2.2	154
18	1.5	168	2.0	170
19	2.0	107	8.3	119
20	—	142	—	165
21	2.3	120	4.4	183
Arithmetic mean	1.8	141.9 ± 7.0	4.2	193.3 ± 7.4

In the first two series of experiments, the nerve was placed on three stimulating (c, d, e) and two recording (a, b) electrodes. The distal part of the nerve, killed by high temperature (shaded area), was as always, turned toward electrode a. The segment from b to c was so small (4-5 mm), that a plate for tapping the loop of current could not be placed on it. In the first series of experiments the distance from b to c was 4 mm, from b to d—11 mm, and from b to e—30 mm. Subsequently, when the nerve was placed on the electrodes, a strength of stimulation was established which

---

* To decrease the loops of the excitatory current a bridge was also used, as described by Erlanger, Bishop and Gasser (1926).

was 4 times higher than the threshold for one of the points. The stimulation was consecutively produced from electrodes c, d, and e, and the oscillograms of the running potentials of excitation were photographed. In all three cases, the nerve fibers under the recording electrode b conducted impulses from an equal stimulus. The only difference was in that in the
231 first case these impulses travelled 4 mm, in the second—11 mm, and in the third—30 mm.

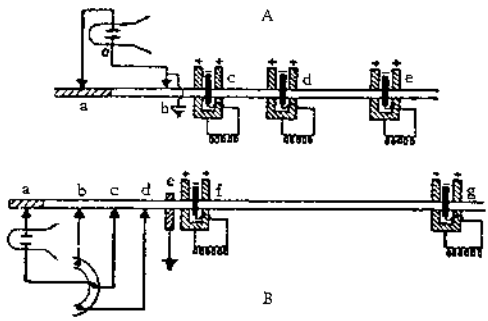


FIGURE 133. Two modifications (A and B) of the apparatus to determine the magnitude of spreading potential, at various distances from the stimulating electrode (according to Averbakh and Nasonov, 1950)
See text for explanation.

The areas of the peaks on the oscillograms were measured by a planimeter and expressed in percentage of the area of the peak which passed the greatest distance (30 mm), and therefore reached a constant value (Table 38). Figure 134 shows three such photographs, successively taken from one and the same nerve. It is clear that the area of the peak A is considerably larger than that of peaks B and C. Also the area of peak B is larger than that of peak C.

The oscillograms clearly demonstrate the loops of the stimulating current. These loops are larger when the stimulating and recording electrodes are closer. The effect of these loops on the dimensions of the areas of the peaks may be very small. As shown in the oscillograms, the phase of the loop directed downward from the peak is in direct proximity to the peak, therefore the loop could only reduce the dimensions of the latter. This being so, the presence of artifacts of the stimulating current may only somewhat diminish the dimensions of the existing decrement.

The results of this series of experiments are summarized in Table 38, where each three successive measurements (at a distance of 4, 11 and 30 mm) indicated by the same number of experiments were performed on a different nerve.

From these data (Table 38) it follows that the impulse arising as a result of a strong stimulus (four times greater than the threshold) exceeds the magnitude of the constant peak by an average of 71% at a distance of

4 mm from the site of its origin. After travelling 11 mm it diminishes, exceeding the regulated peak only by 18%.

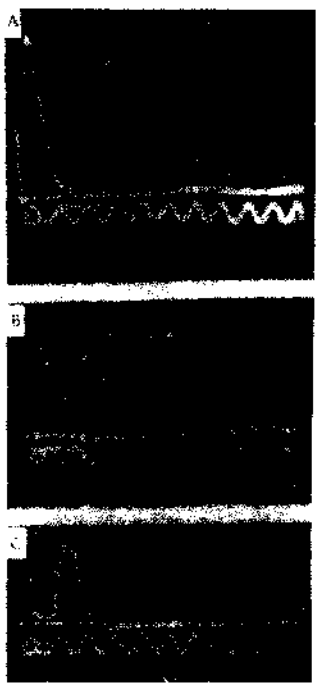


FIGURE 134. Oscillograms of propagation peak, taken at various distances from the site of stimulation (according to Averbakh and Nasonov, 1950)

A—4 mm; B—11 mm; C—30 mm. Time scale: 1 period corresponds to 1 millisecond. Apparatus as in Figure 133, A.

The following series of experiments were performed, with the only difference that the distances between the electrodes varied: between b and c—5 mm, between b and d—15 mm, and between d and e—35 mm (Figure 133, A).

Eighteen experiments on different nerves were performed in all. The results are compiled in Table 39. This shows that the propagating impulse, arising as a result of strong stimulation (4 thresholds), decreases to 139% at a distance of 5 mm from the point of origin, while at a distance of 15 mm it becomes practically equal to the constant peak.

In the previous series it was found that the impulse did not reach constant values even after travelling 11 mm. It exceeded the constant peak by 18%. Consequently, under conditions of these experiments the region of the decrement lies somewhere between 11 and 15 mm.

The apparatus for the third series of experiments is shown in Figure 133, B. Here, near the oscillograph, in addition to the recording electrode A, there are three more electrodes (b, c, d) on the killed segment of the nerve (shaded), each of which may be connected at will by the use of a switch. Further along the nerve there is a grounded silver plate e and two stimulating electrodes f and g, the first of which is placed as close as possible to e, while the other as far as possible, at the very end of the nerve.

In these experiments the distance between the electrodes were as follows: from b to f 17 mm, from c to f—11 mm, and from d to f—6 mm. The distance between the stimulating electrodes f and g was 44 mm.

The experiments were performed in the following manner. After the nerve was placed on the electrodes and its tip, under a, was killed by high temperature, the maximum permissible
233 strength of stimulation, approximately 4 times as strong as the threshold, was given. The recording electrode e, to which impulses were sent successively, at first from f and then from g, was then connected. In the first case the impulse travelled 6 mm, and in the second 50 mm. Both oscillograms were photographed. The first pair of photographs is illustrated in Figure 135, A, B. Both areas were measured and the magnitude of the peak A expressed in percentage of the established peak B originating at a distance. Note that in this series of experiments there were practically no loops of current.

Table 38

Magnitude of potential along the nerve, at various distances from the stimulating electrode (from the site of origin) (according to Averbakh and Nasonov, 1950)

No. of experiment	Relative value of the potential running along the nerve (% of the value of the regulated peak) at the following distance from the stimulating electrode:		
	4 mm	11 mm	30 mm
1	94	127	100
2	116	115	100
3	127	97	100
4	103	97	100
5	166	108	100
6	137	118	100
7	136	100	100
8	133	118	100
9	274	107	100
10	275	114	100
11	133	152	100
12	250	128	100
13	200	119	100
14	310	110	100
15	215	97	100
16	200	125	100
17	213	114	100
18	100	107	100
19	102	103	100
20	180	174	100
21	143	100	100
22	120	118	100
23	204	155	100
24	174	117	100
25	122	140	100
26	178	119	100
Arithmetic mean	171 ± 7.7	118 ± 2.3	100

Next, the recording electrode c was connected (Figure 133, B) and impulses sent to it as in the previous case, from f (a path of 11 mm) and from g (a path of 55 mm). These were photographed (Figure 135, C, D) and the areas measured, the area of the peak C being expressed as a percentage of that of peak D.

The difference between the areas in this case was smaller than between A and B.

Finally, the electrode b to which impulses were also sent from f was connected (path of 17 mm) and from g (path of 61 mm). The oscillograms (Figure 135 E, F) were treated as in the previous cases. No appreciable differences between the areas of the peaks was observed, although peak E

was somewhat higher than peak F but the latter was wider than the former. 234 In this case, only dispersion of the impulses moving at different velocities along different fibers took place (Erlanger, Bishop and Gasser, 1926). Consequently, at a distance b equalling 17 mm, the impulse already approached a constant value.

Table 39

Magnitude of potential along the nerve, at various distances from the stimulating electrode (from the site of origin) (according to Averbakh and Nasonov, 1950)

No. of experiment	Relative value of the potential running along the nerve (% of the value of the regulated peak) at the following distance from the stimulating electrode:		
	5 mm	15 mm	35 mm
1	110	110	100
2	116	100	100
3	125	107	100
4	163	98	100
5	257	100	100
6	177	85	100
7	126	75	100
8	161	111	100
9	104	100	100
10	86	88	100
11	144	122	100
12	159	111	100
13	110	93	100
14	133	93	100
15	149	104	100
16	108	105	100
17	159	97	100
18	114	81	100
Arithmetic mean	139 ± 6.1	99 ± 1.99	100

The data of this series of experiments are shown in Table 40. In this last series of experiments, under the same strong stimuli (approximately 4 thresholds) a quite clear decremental conduction occurred for a distance of 11-17 mm from the site of stimulation.

Analysis of the data from the four series of experiments makes it possible to follow the changes in magnitude of the spreading impulse caused by a supramaximal stimulation, starting from the site of its origin and until the very end of the nerve. Values were obtained for a number of points, each value being an arithmetic mean from 13-26 experiments. These mean values were statistically analyzed and the probable error of the arithmetic mean calculated for each point, indicating the statistical significance of the data obtained. These results are given in Table 41 and illustrated in Figure 136.

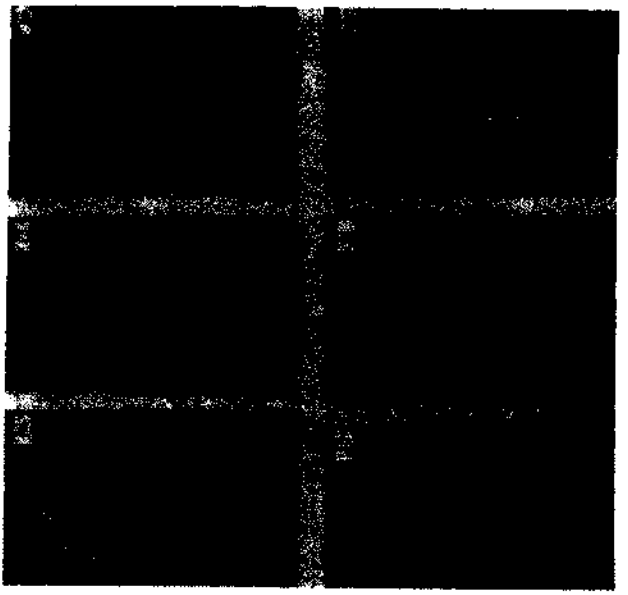


FIGURE 135. Oscillograms of propagation peak at various distances from site of stimulation (according to Averbakh and Nasonov, 1950)

A—6 mm; B—50 mm; C—11 mm; D—55 mm; E—17 mm; F—61 mm. Time scale: 1 period corresponds to 1 millisecond. Apparatus as in Figure 133, B.

From analysis of Table 41, it first of all follows that the data of these different series are satisfactorily corroborated. Thus, for example, determination of the magnitude of local potential (193.3) was performed by quite a different method from the determination of the magnitude of the propagating peak at the nearest point (4 mm). The figure (171.0) shows only a small decrease (from 193 to 171), which is quite understandable, since we are dealing here with propagation with decrement. In this connection it should be noted that the subsequent successive passage of the impulse resulted in a gradual decrease in the value of the potential after each mm. In two cases in which different methods were used (Tables 38 and 40), the
235 value of the potential was determined at one and the same distance from the stimulating electrode (11 mm), and extremely close figures (120 and 118) were obtained. Thus the figures obtained may be considered to be reliable.

The data in Table 41 show that with a sufficiently strong electric stimulation the local electric reaction of the nerve exceeds the value of the established peak, and spreads with decrement until it becomes equal to the

latter. Only after this, does spreading without decrement begin*. Under conditions of our experiments this excess was almost 100%, and the decrement region spread up to $1\frac{1}{2}$ cm.

Table 40

Magnitude of potential along the nerve, at various distances from the stimulating electrode (from the site of origin) (according to Averbakh and Nasonov, 1950)

No. of experiments	Relative value of the potential running along the nerve (% of the value of the regulated peak) at the following distance from the stimulating electrode:			
	6 mm	11 mm	17 mm	50-61 mm
1	147	107	95	100
2	213	—	75	100
3	173	185	100	100
4	118	126	100	100
5	144	136	88	100
6	107	101	93	100
7	113	96	95	100
8	122	110	115	100
9	97	110	94	100
10	185	134	97	100
11	107	94	90	100
12	125	120	120	100
13	131	—	—	100
Arithmetic mean	137 ± 6.6	120 ± 5.2	97 ± 1.7	100

Thus, the assumption postulated by the theory of "gradual" excitation was entirely confirmed experimentally**.

On the basis of this theory and experimental data, a more or less complete picture can now be formed of the interrelationships between magnitude of stimulus, local reaction and propagating impulse (Figure 129).

---

* Our data do not contradict at all the data of Erlanger, Bishop and Gasser (1926) which prove conduction of impulse by the nerve without decrement. These authors began their measurement of the areas of propagation peaks at a distance of 14.5 mm from the stimulating electrode, i.e., exactly from that point where, according to our opinion, the decrement which can be detected by ordinary methods, terminates.

** Similar results were obtained by Schmitt and Schmitt (1940) who experimented with single giant fibers of Loligo. According to them, very strong stimuli may cause a local response exceeding in value the amplitude of the propagation peak. This supramaximal potential spreads with decrement until it becomes equal to the value of the propagation peak. Confirmation of this assumption, according to the authors, is given in their Figure 13. Unfortunately, the reader cannot find any such confirmation from this Figure.

At weak subthreshold stimuli, a local gradual reaction takes place spreading with decrement to a very small area*. At the threshold stimuli, spreading with increment begins up to point c (Figure 129), from which point the impulse moves on without changing its magnitude, due to a special mechanism of autoregulation previously mentioned. According to Hodgkin (1938), the distance of this incremental run is approximately 0.5 cm (the broken line in Figure 136).

Table 41

Changes in magnitude of potential along the nerve, varying with distance from the stimulating electrode of supramaximal stimuli (according to Averbakh and Nasonov, 1950)

Distance from the stimulating electrode	Value of the moving potential (% of the constant one)	Probable error of the arithmetic mean	Number of experiments
0	193.3	± 7.4	21
4	171.0	± 7.7	26
5	139.0	± 6.1	18
6	137.0	± 6.6	13
11	120.0	± 5.2	13
11	118.0	± 2.3	26
15	99.0	± 1.9	18
17	97.0	± 1.7	13
30	100.0	—	26
35	100.0	—	18
50-61	100.0	—	13

It further follows from our theoretical considerations (Figure 129), that if the magnitude of the electric stimulus exactly equals the magnitude of the constant peak, then the potential will spread without decrement from
236 the point of its origin to the very end of the nerve, due to the same mechanism of autoregulation.

Finally, when stimuli exceed the value of the dimensions of the established peak, the local reaction will be greater than this peak (in our experiments almost twice as high); and only upon further passage will it reach the dimensions of the constant peak (at point c in Figure 129). On further spreading, the magnitude of the running peak will be maintained at the same level due to the same mechanism of autoregulation**.

---

* This has been experimentally proven in the experiments by Katz (1937), Hodgkins (1938) and Karaev (1938).

** Vvedenskii stated that certain phenomena connected with the passage of impulses through a narcotized sector may depend on strength of excitation. Recently these statements were confusing to many investigators since according to the "all or none" law, the nerve cannot conduct weak or strong impulses since it always conducts equal impulses ("all"). It has, however, been shown (Nasonov and Rozental', (1952), that at a certain stage of narcosis there is a region of a noticeable decremental propagation in the nerve. The magnitude of the impulse in this case may depend upon the strength of excitation.

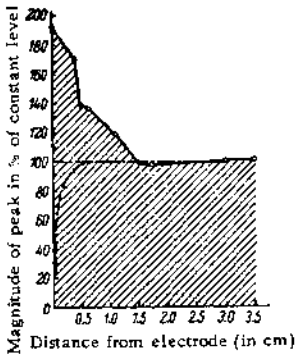


FIGURE 136. Change in area (magnitude) of the potential along the nerve, varying with distance from the point of origin at an above-threshold strength of stimulation. The peak moves with decrement for $1\frac{1}{2}$ cm; subsequently nondecremental conduction is established (according to Averbakh and Nasonov, 1950)

Broken line–incremental run at a threshold strength of stimulation according to Hodgkin's data (1938).

It follows that the value of the established peak is by no means maximal. This is not the "all" required by the "all or none" law. On the contrary, the nerve possesses a more subtle automatic mechanism to regulate the level of the propagating impulse, which in all probability prevents the nerve from being excessively excited, a condition which might cause irreversible changes with the excitation passing into damage of the nerve. The existence of such a mechanism was suggested by Ukhtomskii (1930) in his extremely interesting papers devoted to criticism of the "all or none" law. Thus, both experimentally and theoretically, his hypotheses can be confirmed.

The English physiologists, in their attempts to explain the conduction of an impulse according to the "all or none" law, started from "membrane" concepts, according to which the difference between electric potentials observed after stimulation preexisted in the resting nonstimulated fibers. As already stated, they assumed (Rushton, 1937, et al.) that subthreshold electric stimulus caused only partial, gradual depolarization of the cell membrane; while starting from a threshold intensity, the stimulus suddenly depolarized the membrane completely and all the electric energy preexisting in the fiber was explosively released. According to their theory, something similar to an electric "stopper" takes place in the membrane.

In the light of the above data the very essence of the "all or none" law not only loses
237 its meaning but gives a completely opposite picture of the phenomena in nerve and muscle fibers on excitation, and during formation and spread of an impulse. It therefore seems to us more accurate to use the term "autoregulation of the spreading excitation".

Absolute and Relative Refractory Phases in the Spreading Excitation

It is known experimentally that a stimulated nerve or a muscle fiber is refractory, i.e., nonexcitable or weakly excitable. The membrane theory postulates complete or partial depolarization of the membrane upon stimulation, which interferes with or makes impossible further depolarization, i.e., lowers the excitability of the already stimulated fiber. The protein theory of excitation developed here postulates reversible denaturation of especially sensitive protein molecules of the protoplasm. It is obvious that if such a denaturation took place after excitation, excitability of the fiber would remain lowered until restoration occurred.

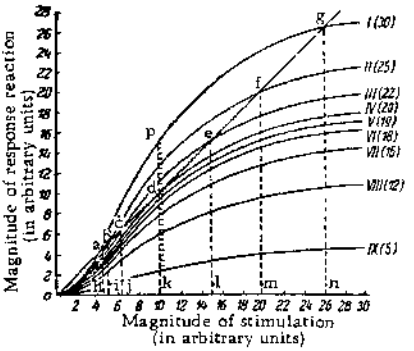


FIGURE 137. Collection of S-shaped curves characterizing excitability of a conducting fiber in different phases of refractoriness or narcosis (according to Nasonov and Rozental', 1952)

The numbers in parenthesis are the values of the limits for the curves. For explanation see text.

It is also known that initially, the stimulated fiber passes a stage of so-called absolute refractoriness, when a stimulus of any strength is not capable of causing a propagating wave of excitation. This stage is suddenly replaced by a stage of relative refractoriness, during which it is possible to cause the appearance of a propagating wave by stimuli considerably exceeding the threshold stimuli of a nonstimulated fiber. Thus, lowered excitability increases gradually with restoration of the original state of the fiber, and either reaches the initial level, or temporarily exceeds it somewhat (hyperexcitation phase according to Vvedenskii). The duration of these stages in nerves of vertebrate animals is measured in thousandths of a second.

The most puzzling thing in the whole picture of recovery of excitability of the nerve is the alternation of the two stages of refractoriness—the absolute and the relative. As far as we know, no attempts were made in the literature to explain this duality. However, these facts seem to contradict the basic assumptions of both supporters of the "all or none" law and their antagonists. Indeed, for the former it would be natural to expect the sudden appearance of initial excitability, i.e., only of the absolute refractory phase; while for the latter it would be more understandable if a gradual recovery of excitability would occur from the very beginning of the process, i.e., existence of only relative refractoriness. However, the obligatory alternation of the two phases, we repeat, is not comprehensible from either point of view.

As will be shown later, the theory of gradual excitation gives an exhaustive explanation of the alternation of the absolute and relative refractory phases (Nasonov and Rozental', 1952).

Let us assume that we are dealing with a nerve fiber, the excitability of which is characterized by curve I (Figure 137). The first intersection a of this curve with the bisectrix will determine the value of the threshold of the fiber (oh). The second intersection (g) will determine the value of the constant peak (ng). Now let us assume that the fiber was stimulated and became refractory, i.e., its excitability decreased. How can decreased excitability be described on the graph? Obviously a new situation exists in the fiber, since it responds to stimuli of the same strength by a weaker response reaction. Consequently, this condition will be described on the graph by an S-shaped curve, all the points of which are located lower than the points of a curve with higher excitability.

Let us imagine that a considerable decrease in excitability takes place, corresponding to curve IX (Figure 137). All the points of this curve lie below the bisectrix and, consequently, stimulation of any strength will cause a response reaction spreading only with decrement. This reaction will rapidly die away, encompassing only a limited segment of the fiber

(local reaction), and therefore conduction without decrement will not take place. In other words, we will have what is really observed during the absolute refractory phase. Further, excitability of the fiber will gradually be restored and can be expressed successively by curves VIII, VII, VI and V. The excitability of the fiber increases continuously, however, until the
239 curve touches the bisectrix. It will conduct only with decrement, and consequently only a local response will arise, i.e., the fiber will still be in a state of so-called absolute refractoriness.

As soon as the excitability curve touches, or only slightly intersects with the bisectrix (Figure IV), the properties of the fiber will at once suddenly change. Starting at this point, the fiber will acquire the ability to conduct excitation without decrement, for any distance whatsoever. Nevertheless, its threshold (ok) is considerably higher than the initial threshold (oh). Here the relative refractory phase begins, in which an impulse spreading without decrement may be obtained, but by stronger stimuli.

Further, the restoration process of the fiber continues, and correspondingly, the curve characterizing its excitability successively acquires positions III, II and finally I. The magnitude of the thresholds diminishes progressively in the process (oj, oi, oh) and the value of the peaks increases (el, fm, gn), until both reach the initial dimensions.

In the presence of an exaltation phase matters do not stop at this point, and the excitability of the fiber may temporarily increase, corresponding to any figure located by all its points above curve I.

It follows that the theory of gradual excitation postulates and explains the whole course of events observed in the experiment, while studying the refractoriness of the stimulated fiber. Moreover, this theory allows certain phenomena to be predicted which were hitherto not observed experimentally. Thus, it has been previously assumed that in continuation of the absolute refractory phase, the fiber completely loses its excitability. From our considerations it follows that at this stage the fiber loses only the ability to create new waves of excitation transmitted without decrement to any distances. At the same time it may respond to stimulation by a local reaction spreading with decrement for a certain distance along the fiber. Therefore it may be forecast that, if at the moment when the maximum peak passes through the fiber, additional stimulation is applied, the value of this peak will increase. Later, on further passage, it will decrease to constant values. We assume that this consequence of the theory may be experimentally checked. This has been indirectly proved by our work with Averbakh (Averbakh and Nasonov, 1950). In any case, if it is true, then the "absolute refractory phase" can in no way be considered absolute, because during this phase the fiber is still capable of responding to stimulations by local reactions spreading with decrement*.

---

* Rosenblueth (1952), who obtained a local response to a strong anodic stimulus applied during the absolute refractory period, came to the very same conclusions.

Absolute and Relative Refractivity on Stationary Stimulation or Narcosis

From the theory of gradual excitation it follows that there is no basic difference between a fast-moving, spreading excitation, and a local, slowly-
240 repaired stable excitation or parabiosis. This concept of the nature of excitation is the basis of Vvedenskii's theory of parabiosis (see also Nasonov and Suzdal'skaya, 1948; Nasonov, 1948b; Grindel' and Rusinov, 1949; Averbakh and Nasonov, 1950).

Indeed, if the excitant acts on any nerve segment for the duration of a relatively long time period, it causes more or less stable, slowly-repaired changes which are called parabiosis by the followers of the Vvedenskii-Ukhtomskii school. Here a stable electronegativity and a state of local narcosis is observed (partial or complete nonexcitability), which may be considered as analogous to refractivity of the spreading stimulus. This condition may be caused by any chemical or physical agent; including the cathode of direct current.

If the electric mechanism of spread of excitation is accepted, it must be assumed that any point of the nerve fiber on passage of an impulse should be subjected to the action of a cathode. Thus it enters a state of parabiosis with all the characteristic features of the latter, including electronegativity and reduced excitability, which we call the "absolute and relative refractory period"*. The only difference between such propagating excitation, and cathodic parabiosis or electronarcosis, will be its extreme shortness. This is explained by the special mechanism of impulse transmission, during which the action of electric current is at once eliminated as soon as the adjacent segment of the fiber is stimulated. If this is so, then the same considerations may be applied to local narcosis of a fiber segment as those proposed for refractivity of the propagating impulse.

Let us assume that the fiber is characterized by excitability of curve I (Figure 137). Its threshold, therefore, equals 'oh' and the value of the propagation peak-ng. We shall subject this fiber to the protracted action of a certain irritant (salt solution, acid, alkali, narcotic agent, high temperature, cathode of direct current, etc.). At the onset of this action, the excitability of the fiber, as shown by the experiment, will increase somewhat and be followed by a continuous decrease. This process should be reflected in the gradual decrease of the S-shaped curve. The latter will successively acquire the positions II, III, IV, V, VI, VII, VIII and IX (Figure 137). Let us see how the properties of the fiber change in the process. It is not difficult to be convinced that here, as in the case of a propagating impulse, two phases sharply divided from each other are observed. Initially, during progressively deepening narcosis, the curve will acquire positions corresponding first to II and later to III and IV. Each of those curves differs from the previous one in that the threshold increases (oh < oi < oj < ok), the

---

* "And thus we find quite a number of similar features in the moving wave of excitation and in the condition of parabiosis which may be considered as a kind of a stationary excitation wave", says Ukhtomskii (1945, p.47). And indeed, let us imagine that we succeeded in stopping a running excitation wave at the moment of maximum peak in the stage of absolute refractivity. Investigation of such a fiber would lead us to the conclusion that it was in a state of deep reversible narcosis caused by the cathode of electric current.

value of the threshold excitation increases (ah < bi < cj < dk), and the amplitude of the propagation peak decreases (gn > fm > el > dk).

This fact, consequent to our theoretical considerations, was experi-
241 mentally observed by Hodgkin (1938) on inhibiting the excitability of a nerve fiber by potassium chloride, and during the relative refractory phase. Hodgkin showed that under these conditions the value of the threshold potential really increases, while the value of the propagation peak falls.

In certain papers the ratio between the value of the propagating impulse and the threshold is described under the title of "safety factor". There are indications that on narcotization of the nerve, the safety factor decreases (Hodgkin, 1938; Katz, 1939; Schmitt and Schmitt, 1940; Pumphrey, Schmitt and Young, 1940). This is directly postulated by our theory. Further, assuming that the peak of threshold potential on the graph lies always on the bisectrix of the coordinate angle, where x = y, it may be postulated that with changes in excitability the value of the threshold excitation should be directly proportional to the value of the threshold potential. This hypothesis, following from the theory, may be quantitatively verified on a single fiber. At the same time, during the whole phase of narcosis the curves still intersect with the bisectrix and therefore, in this period, the capacity of impulse transmission without decrement should still be maintained, i.e., the capacity of excitation, for any distance whatsoever, was determined only by the length of the fiber. This first stage of narcosis corresponds to the relative refractory period, since during this period nondecremental conduction may occur after applying a stimulus of greater magnitude. This period terminates by the position IV of the curve, when the latter touches the bisectrix, and where the value of the threshold becomes equal to the value of the peak and to the value of threshold excitation (ok = dk).

However, as soon as the curve of the narcotized fiber falls even slightly below the bisectrix (Figure 137, curve V), the properties of the fiber should change at once and basically. Such a curve will lie with all its points below the bisectrix and therefore a nerve narcotized to such an extent is able to conduct only with decrement. The conduction is limited to a certain small distance and consequently, from this moment on, local excitation will take place*.

Thus, the postulates of the theory of "gradual" excitation are as follows:

1. On narcotization, the nerve or muscle fiber, similarly to a fiber along which an excitation wave is propagated, should pass through two successive periods of decreased excitability—a relative and an absolute refractivity.

2. During the first relative refractory period, gradual decrease in excitability (increase in thresholds) and decrease in the amplitude of conducted impulse should take place. Nevertheless, the ability of unlimited nondecremental conduction is preserved.

3. The first period should suddenly pass into the second—the absolute refractory period, characterized by conduction with decrement. During this

---

* At the beginning of this chapter it was mentioned that strictly speaking, even a subthreshold excitation of the nerve fiber is a spreading and not a local one, since it is also propagated, but, contrary to the above-threshold one, with decrement. However, a more or less steep decrement determines the limits of spread of such an excitation and provides a reason for designating it as a local excitation.

period, stimuli of any strength may cause only a local reaction and cannot originate a wave of unlimited spreading excitation.

242 It will later be seen, how far these requirements are confirmed by experiments. In this respect, two problems must be solved. The first is that of changing excitability of the nerve with narcosis. The second is that of conduction of an impulse with decrement in a narcotized nerve.

Regarding the first, a great many studies are available (Szpilman and Luschinger, 1881; Verigo, 1899; Vvedenskii, 1901; Dendrinos, 1901; Fröhlich, 1904; Kato, 1924, 1934, and others), but all these studies were technically imperfect. A nerve-muscle preparation was used. The nerve was put through a narcosis chamber into which stimulating electrodes were introduced, and excitability was judged by muscle contraction. After the muscular reaction stopped, excitability was said to have disappeared, although in reality it could only have been said that the impulse could, for some reason, not pass to the nonnarcotized part of the nerve. The method did not permit any assessment of whether the impulse appeared under the electrodes, and what the nature of its spread within the chamber was. In addition, with such a method, a diffusion gradient of the narcotic agent always forms within the nerve, at the borders of the chamber, as a result of which an apparent decrement in conduction may form, due to unequal concentration of the narcotic agent along the diffusion gradient.

In order to avoid these shortcomings, the nerve was totally narcotized in all our experiments, and excitation of its different sectors was judged from oscillograms (Nasonov and Rozental', 1952).

A desiccator of 5 liter total capacity (Figure 138) was used as the narcosis chamber. A glass support was fixed to the stopper in its lid (b), and two pairs of electrodes (c-excitatory and e-recording) and a grounded plate d were attached to it. The distance between electrodes c and e was 4.5 cm. An isolated frog's sciatic nerve was placed on the electrodes and on the plate. The proximal end of the nerve was stimulated; 100 ml of Ringer's solution were poured into the lower part of the desiccator (a). The rim of the desiccator was smeared with vaseline, the desiccator was closed, and the nerve was thus kept in a moist chamber. For 3-6 minutes the thresholds of excitability of the nerve were determined by the first noticeable disturbances of the horizontal passage of the ray on the oscillograph screen.

After this the lid of the desiccator, together with electrodes and nerve, were lifted and the Ringer's solution was replaced by the same amount (100 ml) of solution containing the narcotic. The lid was replaced. The narcotic vapors began to fill the space of the chamber and the nerve was gradually narcotized. During this time the thresholds were determined at one minute intervals, up to the complete disappearance of any traces of nervous activity on the oscillograph at maximal stimulation. One-two minutes later, the lid (with the electrodes) was again lifted and transferred to another desiccator into which pure Ringer's solution was poured. From that mo-
243 ment restoration of nerve excitability started, which, as before, was accompanied by determinations of the thresholds at one minute intervals and by photographing the oscillograms.

Solutions of 0.5–2% chloroform, 10% ethyl alcohol, 1.5% ethyl ether, and 0.4% ammonia were used as narcotics*.

---

* For ammonia weight concentrations are given, while for the other substances, volume concentrations are given.

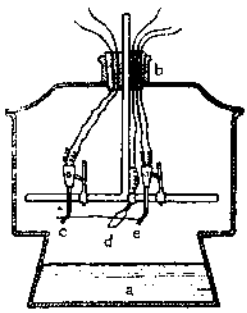


FIGURE 138. Chamber for total narcotization of the nerve, made from a desiccator (according to Nasonov and Rozental', 1952)

244 a—Ringer's solution or a solution of a narcotic; b—lid of the chamber, through the rubber stopper of which passed the holding handle of electrodes and wires; c—excitatory electrodes; d—grounded plate; e—recording electrodes.

The excitatory electrodes were removed from the recording electrodes as far as the length of the nerve allowed (4.5 cm). This ensured that the electrodes recorded only the nondecrementally conducted effect, because, as will be seen later, local excitation spreading with decrement encompasses much shorter segments of the nerve. The results of these investigations are illustrated in Figure 139.

Six to ten experiments were performed with each narcotic. They all gave the same results and therefore, in Figure 139, only some of them are given.

The experiment with 2% chloroform may be considered typical (Figure 139, A). Here, immediately after placing the nerve in the narcotic vapors (first arrow), a transitory slight rise in excitability (20%), lasting about 1.5 minutes, was observed. This is the stage of increased excitability according to Vvedenskii. This was observed after the action of all the agents tested, with the exception of ether. Furthermore, narcotization of the nerve was sharply divided into two stages. At the beginning, excitability decreased quite rapidly, but on checking excitability at one-minute intervals the graduality of this process was readily seen. At the ninth minute of narcosis, when excitability decreased to 55%, it suddenly dropped to zero. This transition from gradual change to absolute disappearance of conducting effect is as postulated by our theory. It corresponds to a drop in the curve of excitability below the bisectrix (from curve IV to curve V, see Figure 137) and to the transition from a nondecremental conduction to spreading with decrement, which cannot be observed at such a far distance. Our instrument allowed for registering of manifold increases in excitation; however, no stimuli could now cause conduction without decrement. This is the absolute refractory period*.

Two minutes after the onset of this period, the narcotic action was eliminated (second arrow), and the threshold was checked at 1 minute intervals. As seen from Figure 139, A, excitability returned at once with a similar sharp peak as seen at its disappearance before, and to a considerable level, very close to the initial one (about 55%). This also agrees with the requirements of the theory. At that moment, the excitability curve again touched the bisectrix (Figure 137, curve IV) at the same point at which it departed. At this stage the absolute refractory period is replaced by the relative refractory period. During the latter, conduction effect may be obtained, but for this a stronger excitation is required. Later, excitability

---

* It will be shown later that during this phase the nerve really responded to local excitation, spreading with decrement, which may be recorded by the electrodes located near the site of stimulation (see Figure 148).

gradually increased and finally reached the initial level. Consequently, the recovery curve is characteristic in that it does not depart gradually from the zero level but rises almost vertically.

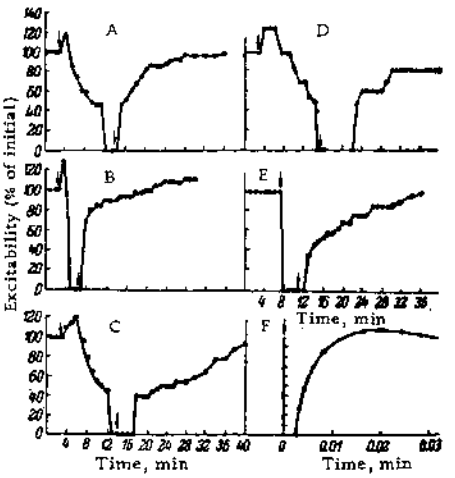


FIGURE 139. Change of excitability of nerve after narcosis and restoration (according to Nasonov and Rozental', 1952)

A, B—2% chloroform; C—10% ethyl alcohol; D—ammonia; E—1% ethyl ether; F—change in excitability of the nerve on spreading of an impulse (according to Lucas, 1913). The arrows designate beginning and end of narcotization.

In Figure 139, B, a common situation is described, where after a short increase in excitability the nerve rapidly enters a state of narcosis. In these conditions, on transition to narcosis it is difficult to differentiate between the relative and absolute refractory periods, but the dividing line between these periods is very clearly seen during repair. The same situation occurs after the action of ether (Figure 139, E), but here the stage of increased excitability is entirely absent. It is highly probable that this stage is not observed here because the process of narcotization is too rapid*.

In the process of repair, excitability not only reaches the initial level but somewhat exceeds it (Figure 139, B). This frequently observed phenomenon may be compared with the exaltation phase of Vvedenskii, which the British authors call super-normal.

---

* Fröhlich (1904) was also unable to demonstrate a stage of increased excitability with ether narcosis, while a number of authors had previously observed it with other narcotics (Efron, 1885; Gad, 1888, 1889; Vvedenskii, 1901 and others).

Figure 139, C, D, illustrates narcotization by 10% ethyl alcohol and by ammonia vapors. The same stages of narcosis occur as with chloroform. First there is a state of increased excitability (especially manifest
245 due to NH_3), followed by a relative refractivity characterized by a gradual decrease in excitability. Later, there is a sudden complete disappearance of the effect, corresponding to the absolute refractory period. Here, as in the case of chloroform, restoration of excitability occurs suddenly, with a "jump", approximately to the initial level, followed by gradual return to normal.

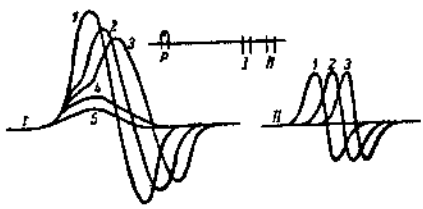


FIGURE 140. Scheme of conduction of a nerve impulse through a narcotized sector in an isolated nerve fiber (according to Bullock and Turner, 1950)
For explanation see text.

In our opinion, there is no basic difference between the phenomena observed when the nerve fiber emerges from the refractory state during stable and propagating excitation. The difference is purely quantitative. In the first case, the action of the excitant is a protracted one, and the phenomenon itself lasts tens of minutes and even more. In the second case, the action of the agent is very short-lived and relatively intense, and therefore the entire process is measured in milliseconds.

Figure 139, F, shows (for comparison) a curve of the change of excitability of the nerve on emerging from the excitation state. It is not difficult to see the similarity between this curve and the picture of restoration after narcosis (compare Figure 139, E and F). The whole process of repair is also divided into absolute and relative refractory periods. Here the curve of excitability also rises almost perpendicularly to the abscissa, and does not depart from it gradually as might have been expected. And, finally, here too, as in the case of narcosis, excitability is sometimes restored only to the initial level, but in certain cases exceeds the latter, passing into a state of hyperexcitability. Previously it had been shown that in both cases these similar phenomena can be given the same explanation.

Recently, independently of us, and on the basis of purely empirical data, Bullock and Turner (1950) came to the same conclusions, working with isolated giant nerve fibers of the earthworm Lumbricus terrestris, Marphysa, and the decapod Panulirus. Figure 140 schematically illustrates their experiment, where in addition to the excitatory electrodes (P) there are a pair of tapping electrodes I, and at a certain distance from them a pair of tapping electrodes II.

In the region of the electrodes I the nerve was subjected to the action of various agents which inhibit excitability (narcotics, reexcitation, direct current). The level of the peak changed with time in the manner described above. At first a gradual decrease in its amplitude (I, 1-3) was seen, but conduction of the impulse to the distal electrodes of the nonnarcotized region
246 was preserved (II, 1-3). This phase corresponded to the relative refractory phase of narcosis. On further deepening of narcosis the picture changed sharply, after the value of the peak decreased by 25% from the initial value. Conduction of the impulse to the distal electrodes (II) ceased and only a local effect was seen in the narcotized region (I, 4, 5), and which gradually faded away

Thus, the requirements of the theory concerned with changes in excitability of the nerve on narcotization were fully confirmed.

Conduction of Impulse with Decrement in a Narcotized Nerve

The previous chapter discussed in detail the question of how, from the point of view of the theory of gradual conduction, narcosis would affect the excitation wave passing along the nerve. It has been said that the theory postulates two successive phases of action of the narcotic. In the first phase (relative refractory period) narcosis only diminishes the amplitude of propagating impulse and increases the threshold (lower excitability), whereupon spreading will occur without decrement. If only a sector of the nerve is narcotized, then the impulse that has reached this sector through the normal fiber should decrease in amplitude on entering into the narcotized region, but will continue to spread without decrement. On emerging from the narcotized region, the amplitude of the impulse will return to normal, corresponding to the impulse of the normal fibers.

On further deepening of narcosis this first period abruptly passes into the second one—the absolute refractory period. At this stage the nerve at once loses its ability to conduct impulses without decrement. While moving, the value of the impulse rapidly decreases and only a small limited area is involved in the excitation. The nerve loses its ability to conduct excitation without decrement, and may respond only by a local reaction to any stimulus, no matter how strong.

However, a sharp jump from one phase to the other may be expected only in the case of an isolated fiber. Working with a nerve consisting of many fibers, this transition should be more gradual, since in the intermediate stages of narcosis some of the fibers easily succumb to the effect of the narcotic. They start to pass into the absolute refractory period and conduct, at the beginning with a mild, and subsequently with a more and more steep decrement. This gradual admixture of less excited fibers creates the impression of a gradually increasing decremental conduction in the entire nerve. With deeper narcosis all the fibers should conduct the stimulus with a more or less steep excitation decrement.

This is what could be expected theoretically. What is observed in fact?

At the turn of the century, numerous papers appeared in which the authors (in the majority of cases on the basis of indirect evidence), supported the theory that conduction of impulses, in the narcotized nerve, occurred with decrement (Szpilman and Luschinger, 1881; Dendrinos, 1901; Boruttau and Fröhlich, 1904; Lodholz, 1913; Lucas, 1913, 1917; Verworn, 1914; Vorontsov, 1931; Rezvyakov, 1930, 1934). The main argument cited in favor of this theory was the observation made by a number of investigators, that under otherwise equal conditions, a long sector of the nerve is more rapidly narcotized than a short one (Szpilman and Luschinger, 1881; Verigo, 1899;
247 Fröhlich, 1904 and others).

Besides this main concept others were also postulated. Thus, Boruttau and Fröhlich (1904) showed that when a nerve was narcotized, there was always a greater negative oscillation of the current of rest in the region closer

to the excitatory electrode, than in the more distal sectors. Lucas (1913) postulated that change in the interval of summation in different sectors of the narcotized nerve indicates the existence of an excitation decrement. Finally, in order to confirm the presence of decrement during narcosis it was said that the narcotized nerve which ceased to respond to excitation began again to react when stimulus intensity was increased. That in this case the action of current loops was not a contributing factor was proved by the fact that the effect ceased on constriction of the nerve, or on pressure applied to it.

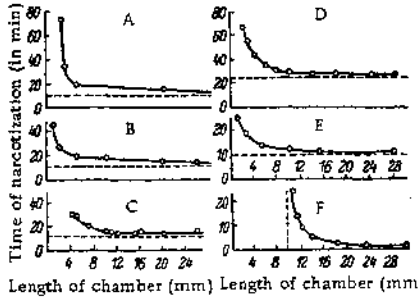


FIGURE 141. Relationship between the limit length of the narcotized segment of the nerve, and duration of narcotization

A—ethyl alcohol (according to Verigo, 1899; B—ethyl alcohol (according to Fröhlich, 1904); C—temperature of 37° C (according to Makarov, 1932); D—mechanical pressure (according to Makarov, 1939); E, F—theoretical curve (according to Nasonov and Rozental', 1952).

248

It seemed that the fact of decremental conduction of an excitatory impulse by a narcotized nerve was widely accepted. However, this question was revised after a series of investigations by the Japanese physiologist Kato (1924, 1934) and his co-workers.

Kato is an extreme supporter of the theory, in our opinion an erroneous one, that excitation occurs entirely according to the "all or none" law. Therefore, a priori, he denied the possibility of decremental conduction, mainly on the grounds that such a conduction basically contradicts the above law (Kato, 1924). He attempted to prove his point by many ingenious experiments on isolated nerve fibers (Kato, 1934).

The influence of Kato's works on physiologists was so marked, that some of the followers of the "decremental" theory of conduction retracted their previous opinions in print! (Davis, Forbes, Brunswick and Hopkins, 1926). The solution to the problem of impulse conduction during narcosis was thought to be the concept of nondecremental conduction (Heinbecker, 1929; Lanczos, 1930; Davis, Forbes, Brunswick and Hopkins, 1926; Tasaki, 1939 and others).

Among Vvedenskii's followers there is no agreement on this problem. While Vorontsov (1931) and Rezvyakov (1930, 1934) express themselves in favor of the decrement theory, Ukhtomskii (1927) (if only on the basis of theoretical considerations), rejected decremental conduction. Makarov (1932) cites data both in favor of and against the two opposite points of view.

What then are the arguments against decremental conduction during narcosis?

One of the most convincing studies in favor of decrement was one dealing with the effect of the length of the narcotized nerve segment on the speed of onset of nonconductivity in this segment. Verigo (1899) studied this relationship in a nerve narcotized by alcohol and chloroform vapor, and he was the first to express it graphically in the form of a curve

(Figure 141, A). Later, the same relationship was studied by Fröhlich (1904) for the action of ether on the nerve of a frog. Figure 141, B shows a curve plotted from his data. Makarov (1932, 1939) studied the effect of the length of the nerve segment on speed of onset of nonexcitability under the influence of high temperature (37° C). Figure 141, C shows a curve plotted from his data. In addition, Makarov observed the same results under the influence of mechanical pressure (by plates of various lengths) on the nerve (Figure 141, D).

The four cases mentioned are characterized by very similar curves, the two lines of which approach asymptotically 2 mutually perpendicular straight lines. The lower asymptote is parallel to the abscissa, located at a distance of 10 minutes from it*.

Verigo (1899) drew attention to the fact that a simple inversely proportional relationship between the length of the segment and duration of narcosis is not correct, and that this relationship is obviously more complicated. The only fact which in his opinion may be deduced is that the length of the nerve segment considerably affects the speed of narcotization of short segments (approximately up to 5 mm), this dependence being extremely slight further on. Makarov (1932, p.136), stated this dependence as follows: "Experiments with heating various lengths of the same nerves of one frog have shown that if the heated areas of the nerves were not longer than 8-10 mm, conductivity disappeared later, the shorter the heated area. However, increase of the heated area over 10 mm did not affect the time of disappearance of conductivity.

Finally, Kato (1924, 1934), experimenting with isolated fibers and with whole nerves, observed the same regularity. Working with small segments of a nerve (in chambers less than 6 mm in length) the dependence of speed of narcosis on the length of the segment was clearly defined, but as soon as Kato used larger segments (chambers longer than 1 cm) conduction ceased independently of the length of the nerve segment. Since Kato
249 was an extreme supporter of the "all or none" law he was obliged at all costs to refute the existence of decrement during narcosis. That is why he was also forced to refute the relationship speed of narcosis to the length of the nerve segment. He considered this dependence to be an artifact, explained by the fact that in small chambers the diffusion gradient of the narcotic substance affects the nerve throughout its entire length. It should be said, however, that even according to his supporters such as Davis, Forbes, Brunswick and Hopkins (1926), Kato presented no direct confirmation of this explanation. But at the same time, his further experiments which were aimed at refuting the theory of decremental conduction were performed with larger nerve segments, i.e., in the region where according to the data of Verigo (1899), Fröhlich (1904), Marakov (1932) and others, the dependent relationship between the time of narcosis and length of the nerve is almost nonexistent.

---

* In the paper by Verigo (1899) the upper branch of the curve (Figure 141,A) approaches asymptotically a certain straight line parallel to the ordinate, removed from it by approximately 2 mm. However, this was not confirmed by later studies, and as shown in Figure 141, the upper branch of the curve approaches asymptotically directly to the ordinate axis, in the data of all the other authors.

These data have been analyzed in detail, since they were the source of Kato's main error. He assumed that in those cases where it occurs, decrement during narcosis was related to the diffusion gradient of the narcotic substance at the edge of the chamber.

How can this peculiar regularity be explained, according to which the relationship between the speed of narcosis and the length of the nerve segment is readily observed in short segments and is practically nonexistent in long ones?

Makarov (1932) postulated that these phenomena seemingly contradict the ideas of decremental conduction of an impulse in a narcotized segment. We shall attempt to show that no such contradiction exists.

We will attempt to describe the series of changes in the value of the impulse peak on passage through a nerve segment in varying stages of narcosis. This may be done quite objectively, starting from the two basic postulates, namely, that the electric response reaction of the nerve is always gradual and may be expressed by an S-shaped curve (Figure 137), and that spread of impulses in the nerve occurs by an electric mechanism according to the principle of "small currents"

Figure 142 shows a schematic illustration of a narcosis chamber through which nerve ab is drawn. It is assumed that the whole nerve segment within the chamber is evenly narcotized without a diffusion gradient at the edge of the chamber, and that each nerve fiber becomes gradually narcotized, successively passing the stages characterized by excitability curves from I to IX (Figure 137).

According to the theory of "small currents", any nerve segment on being stimulated becomes electronegative in relation to the adjacent nonstimulated segment. Due to this fact, an electric current arises, closing the nerve outside in the direction from nonstimulated to stimulated segment. This current stimulates the resting segment, as a result of which the latter becomes electronegative in relation to the following nonstimulated one and, in its turn, stimulates the latter, and so on.

Let us now imagine that an impulse was propagated along a normal nerve, reaching the border of the chamber. The excitability of such a nerve is characterized by curve I (Figure 137), and the value of its peak (ng) will equal 26.3 arbitrary units. Let us assume that the nerve segment (Figure 142) is narcotized to a degree corresponding to curve V (Figure 137). This curve is given separately in Figure 143. It lies below the bisectrix and therefore the impulse should spread with decrement. Since the value
251 of the peak of the last healthy segment is 26.3, the first narcotized segment is subjected to a stimulation equal to 26.3. In order to find the response reaction of this segment, we take 26.3 on the abscissa and on the ordinate find the value of the response reaction, which is 16.5 (Figure 143). This will be that intensity of electric response which will be developed by segment I (Figure 142), acting on the adjacent segment 2. In order to find the response reaction of this segment we take the value of 16.5 on the abscissa of the curve (Figure 143) and on the ordinate find the value of 13.7. In a similar way we find the value for each segment: for 3—12.1, for 4—11.1, for 5—10.4, for 6—9.8, for 7—9.3, for 8—8.8, for 9—8.3, for 10—7.9, for 11—7.3, for 12—6.7, for 13—6.0, for 14—5.2, for 15—4.1, for 16—2.8, for 17—1.3, for 18—0.4 and finally for 19—0.1.

Taking successive segments on the abscissa of Figure 142 and the corresponding values of response reactions on the ordinate, we construct

curve V, describing the change in value of the electric peak on passage of an impulse with decrement along the narcotized nerve segment. As seen from Figure 142, such an impulse will not leave the chamber, fading away in it. The curve of this fading away is extremely characteristic. At the beginning, the value of the peak falls rapidly, subsequently forming a certain plateau, and finally it steeply approaches the abscissa. This is the curve of an impulse in the decremental stage of narcosis (absolute refractory period).

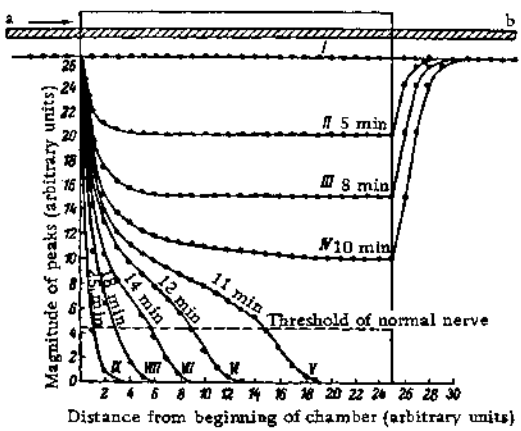


FIGURE 142. Theoretical curves of change of the value of the impulse after its passage through a narcotized nerve segment (according to Nasonov and Rozental', 1952)

Rectangle—schematic illustration of narcotization chamber; ab—conducting fiber. For other explanations see text.

In a similar way the curve of the impulse is constructed in the stage of nondecremental conduction during narcosis (relative refractory period). Let us imagine that the nerve segment is narcotized to a condition corresponding to curve III, Figure 137. This curve is illustrated separately in Figure 144.

We already know that an impulse reaching the chamber by propagation along a healthy nerve has a peak of 26.3, and therefore this will be the value of excitation of segment 1 of the narcotized nerve. Taking 26.3 on the abscissa of Figure 144, we find the value of the response reaction of this segment to be 19.2. Figure 144 shows the segment of the curve located below the bisectrix; consequently, further spread of the impulse should occur with decrement, until the impulse will reach the point of intersection of the curve with the bisectrix. Therefore using the same method of graphical determination of the value of the moving peak we find that segment 2 will give a smaller value—17.3, segment 3—16.3, segment 4—15.7, segment 5—15.4, segment 6—15.2. The subsequent ones will, by infinitesimal decrease, approach the constant value 15.0, corresponding to the point of intersection of the curve with the bisectrix. Further on, the peak will be maintained at

252 the same level and curve III (Figure 142) will run practically parallel to the abscissa. Thus, a nondecremental spread of impulse on a lower level will be achieved inside the narcotic chamber (see curve III, Figure 142).

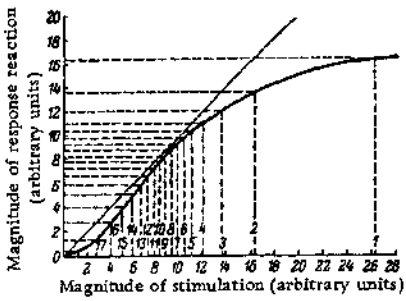


FIGURE 143. Excitability curve (curve V, Figure 137) according to which a theoretical curve of change of the value of the impulse (curve V, Figure 142) is constructed (according to Nasonov and Rozental', 1952)

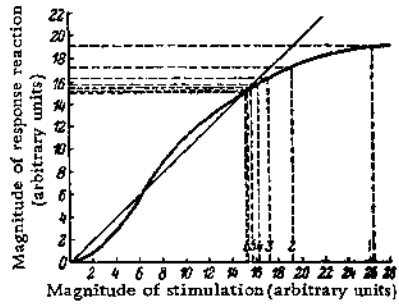


FIGURE 144. Excitability curve (curve III, Figure 137) according to which the theoretical curve of change of value of the impulse (curve III, Figure 142), is constructed; (according to Nasonov and Rozental', 1952)

Having reached the border of the chamber (segment 25) at a constant level of 15.0, the impulse will begin to stimulate the nearest normal nerve segment located outside the chamber (segment 26). The excitability of this segment is characterized by curve I (Figure 137). This curve is illustrated separately in Figure 145. A stimulus of value 15.0 will give here a response reaction of 20.3, whereby we arrive at a segment of the curve located above the bisectrix and consequently obtain an increment in impulse. Indeed, a stimulus of 20.3 will cause a response of 24.2, the latter will give 25.8, then 26.2 and subsequently a series of values will follow, infinitesimally approaching the constant value of 26.3—the point of intersection of the curve with the bisectrix. The course of this rising segment of curve III is described in the righthand part of Figure 142. It can be seen here that after leaving the chamber the impulse starts to move with a steep increment until it reaches its initial level which is 26.3.

Using this graphic method, curves of the change of value of propagating impulses for all those stages of narcosis may objectively be constructed with excitability curves described in Figure 137 (from I to IX).

Using the above graphic method a curve of change may be constructed of the peak, after above-threshold and subthreshold stimulation of the normal nerve. Figure 146, A shows an S-shaped excitability curve of the nerve, whose threshold is 5.0. Upon stimulation equal to 5.2, a response of 5.4 occurs in segment 1. Stimulation of 5.4 will cause a response of 5.8 in segment 2, etc. According to these numbers the curve a in Figure 146, B, is constructed, describing the initial change of the value of the spreading peak following an above-threshold stimulation.

Subsequently, a subthreshold stimulus (4.8) is given, and a curve of decremental spreading, of a subthreshold local reaction (b) is thus obtained.

At the beginning of curve a, a certain latency is seen with a small increase in the peak and then a steep rise to the value of the constant peak (= 20). At the beginning of this chapter it was mentioned that Hodgkin (1938), studying the appearance of excitation in the nerve fiber of the crab observed, purely empirically, phenomena which are exactly reproduced by our theoretical considerations. Following above-threshold stimulation, the excitation arising in Hodgkin's experiment also passed through a short latency at first.
253 After this the excitation rapidly increased, reaching constant dimensions of the peak (Figure 146, C, curve a). Subthreshold excitation, as in our experiments, spread with a decrement (Figure 146, C, curve b).

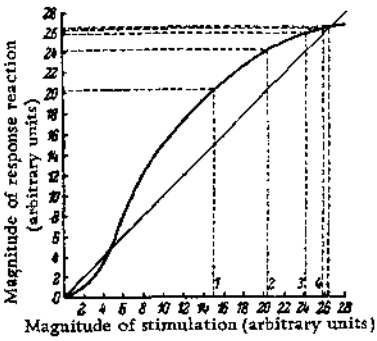


FIGURE 145. Excitability curve (curve I, Figure 137) according to which the rising part of curve III, Figure 142, is constructed (according to Nasonov and Rozental', 1952)

In Figure 142 two phases sharply divided from each other are seen. In the first phase (from I to IV) the impulse fell steeply to a certain level and continued without decrement. Leaving the chamber, it increased steeply to the initial level and spread further without change. On further deepening of narcosis, the course of the impulse changed sharply and the second phase followed—spreading with decrement (from V to IX). The first phase at once passed into the second, as soon as the excitability curve on Figure 137 fell below the bisectrix.

Figure 142 shows that impulses moving in accordance with curves V-IX fade before reaching the borders of the chamber. However, it is easy to understand that in shorter chambers even an impulse spreading with decrement may turn out to be an above-threshold one at the end of the chamber and then, upon leaving the chamber, will begin to move with increment to its initial level.

Regeneration of this kind of impulse which previously decreased in the region of narcosis, after its exit into the normal region of the nerve, was, in fact, observed by Bullock and Turner (1950) on giant isolated nerve
254 fibers of certain worms and crustaceans.

If a dotted horizontal line is drawn on Figure 142 at the level of the threshold (in our case 4.5) it is not difficult for each of our curves (from V to IX) to find a length of a chamber at which the impulse will not fade but will be able, after leaving the chamber, to increase back to the initial value and to move on further without decrement. This length is determined by the intersection of the curves with the horizontal level of the threshold. An impulse running according to curve V will be able to pass through a chamber not longer than 14.7. For curve VI the limit of the length of the chamber is 8.9, for curve VII—5.5, for curve VIII—2.7 and finally, for curve IX—1.0.

This is how our theory explains the relationship between depth of narcosis and length of the narcotized segment of the nerve. The majority of investigators express the former by the time limit of narcotization during which the impulse can still pass through a chamber of a certain length.

They give dependence curves for this time related to length (Figure 141). We can also construct such a curve.

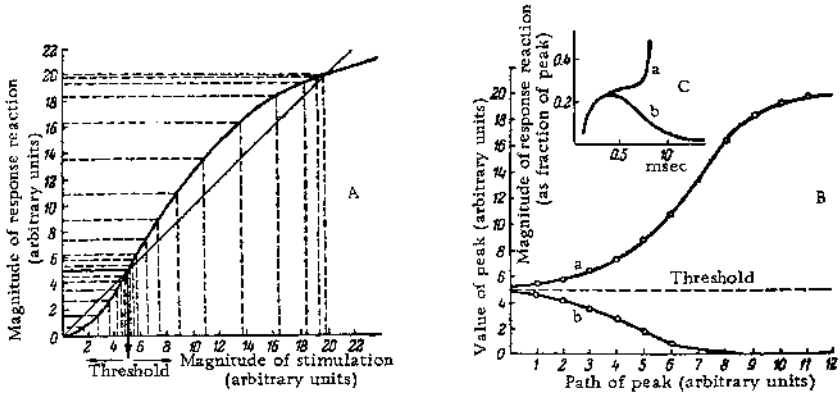


FIGURE 146. Construction of spreading curves of above-threshold and subthreshold impulses

A—theoretical curve of excitability of the conducting fiber. The vertical broken lines starting from the threshold (5.0) correspond to a number of successive values of the spreading excitation (peak) from one segment of the fiber to the following one. To the right of the threshold there are values of the increase in excitation (increment) after excitation by a stimulus exceeding the threshold by 4% (5.2). To the left of the threshold are the values of decreasing excitation (decrement) after excitation 4% below the threshold (4.8).
B—theoretical curves drawn on the basis of the previous data: a—increment of spreading excitation after an above-threshold stimulation (5.2); b—decrement of spreading excitation after subthreshold stimulation (4.8).
C—curves from the paper by Hodgkin (1938): a—oscillogram of incremental spread of excitation as a result of an above-threshold stimulation; b—oscillogram of a decremental spread of excitation as a result of a subthreshold stimulation (A, B, according to Nasonov and Rozental', 1952).

Let us imagine that deepening of narcosis takes place more or less regularly, and accordingly the excitability curve (Figure 137) decreases in a unit of time by more or less the same distance. In Figure 137 such a scale is given in the form of the calibrated segment rl. Each division of the scale will be arbitrarily designated as a minute. In that case, on gradual deepening of narcosis, curve I will occupy the position of curve II after 5 minutes, of curve III after 8 minutes, of curve IV (tangential to the bisectrix) after 10 minutes. After that, spreading with decrement will begin. After 11 minutes the curve will occupy position V, after 12 minutes position VI, after 14 minutes position VII, after 18 minutes position VIII, and finally, after 25 minutes position IX. We have already seen that the limit length of the chamber through which an impulse moving according to curve IV may

pass is infinity, for curve V it is 14.7, for curve VI--8.9, for curve VII--5.5, for curve VIII—2.7 and finally, for curve IX—1.0.

On the basis of these data curve E may be constructed on Figure 141, with a significance identical to the 4 other empirically obtained curves presented in the same figure. It has been mentioned above that, according to P.O. Makarov, these curves contradict the hypothesis of decremental conduction of an impulse during narcosis. However, we have just seen that not only is there no contradiction, but, on the contrary, the decremental theory postulates such a curve and consequently is completely confirmed by the experiment.

Figure 141 shows that the lower branch of the 4 empirically obtained curves does not approach zero but to a certain line parallel to the abscissa, removed from it by a certain distance. The meaning of this will be understood if the theoretically obtained curve (E) will be presented in the form of curve F (Figure 141), where the abscissa indicates time, and the ordinate—the length of the narcotized segment. Curve F shows that during the first ten minutes of narcosis the length of the narcotized segment of the nerve through which an impulse may pass equals infinity. In other words, this indicates nondecremental conduction (period of relative refractivity). After 10 minutes a second period sets in immediately, during which the impulse may pass only through a chamber of a given length (decremental conduction characteristic of the absolute refractory period).

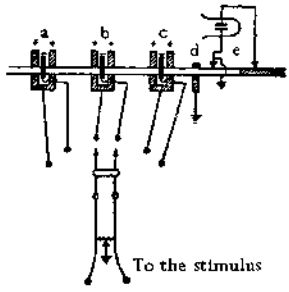
255 Thus, on purely theoretical considerations we come to the conclusion that, given time, a narcotized nerve fiber should pass through two stages, the first of which is characterized by nondecremental conduction with a progressively diminishing amplitude of the peak, while the second is characterized by conduction with a progressively increasing decrement. However, a clearcut transition between these two stages may be expected in its pure form, while working either with a single fiber or with a nerve of a more or less homogenous composition of fibers. If, however, we consider a mixed nerve consisting of fibers markedly differing from each other in sensitivity to the narcotic, then at the early stages of narcotization different fibers will start to pass into the decremental stage. As a result, at the beginning, weakly expressed signs of decrement are observed, becoming increasingly stronger with time. The experiment completely confirmed our assumptions (Nasonov and Rozental', 1952).

The sciatic nerve of a frog was used in the experiment. An isolated nerve was placed on 3 pairs of stimulating electrodes, a, b, c (Figure 147), on a grounded plate d and on a pair of recording electrodes e. Each pair of stimulating electrodes consisted of a fork-like plate of the anode, in the middle of which a wire-like cathode was placed. The two poles were connected by a resistor on which a grounded indicator was moving. These electrodes described by Averbakh and Nasonov (1950), together with the grounded plate, entirely eliminated the current loops on the oscillograms. The distance between neighboring cathodes of the stimulating electrodes, and between the electrode c and the extreme recording electrode, was 15 mm*. All electrodes with the nerve lying on them were placed inside a desiccator, which served as a moist chamber for narcosis (see Figure 138). The strength of stimulation in all cases was such that further strengthening did

---

* In experiment no. 1 the distance between each pair of stimulating electrodes was 17 mm and in experiment no. 2–20 mm.

not cause a noticeable increase of the peak. The nerve was totally narcotized, which entirely excluded the presence of a diffusion gradient of the narcotic.



256 FIGURE 147. Scheme of apparatus for placing the electrodes inside the chamber for the study of decremental conduction during narcotization of the nerve (according to Nasonov and Rozental', 1952)
a, b, c–distal, middle and proximal stimulating electrodes; d–grounded plate; e–recording electrodes.

The experiments were performed in the following manner. The nerve, placed on the electrodes, was introduced into the moist chamber and the thresholds of excitability were determined until they became constant. Subsequently, the oscillograms of the impulses arriving from the (c) proximal, (b) middle and (a) distal electrodes were photographed. Later, 200 ml of 9% ethyl alcohol were poured on to the bottom of the chamber. Usually, narcotization under these conditions in alcohol vapors is so fast that the oscillograms cannot be photographed in the course of the developing narcosis, and the study has to be limited to the pictures of restoration from narcosis*. Subsequently, at various time intervals from the beginning of narcotization, pictures of the oscillograms were taken of the peaks arriving successively from the 3 stimulating electrodes, and the threshold of excitability from the distal electrode (a) was simultaneously determined. Narcosis was stopped by transferring the lid of the vessel, with all the electrodes and the nerve lying on them, to another vessel of the same dimensions, on the bottom of which 100 ml of pure Ringer's solution was previously poured. Study of the process of nerve restoration was conducted in the same way.

Subsequently, precise measurement was carried out by a planimeter of all the areas of the peaks on the oscillograms. The results of these experiments are shown in Table 42. In all, 14 such experiments were performed and 86 series of oscillograms, each from the 3 electrodes, were prepared and measured.

From Table 42 it first of all follows that before narcosis the areas of the peaks from the proximal, middle and distal electrodes differ from each other in the direction of larger or smaller values, by not more than 10%. This is approximately the accuracy of our method. Very soon after beginning of narcotization a sharply expressed decrement of conduction appears, which in the majority of cases considerably exceeds the limits of probable error and is well observed both at the beginning and toward the end of the narcotic stage.

Figure 148 illustrates the stages of narcotization of one frog nerve. In the first horizontal row of the left hand half, oscillograms are shown which were taken before narcotization, from the middle and the distal electrodes (the oscillogram from the proximal electrode was spoilt). Notwithstanding the fact that peak a was 15 mm lower than peak b, its area equals the latter (with 5% difference, which is within the limit of

---

* In experiments nos. 2 and 14 we succeeded in obtaining the complete picture of both entry into and exit from the narcotic condition.

experimental error). The decrease in its height is compensated by the widening of its base (dispersion of velocities of movement of impulses in different fibers).

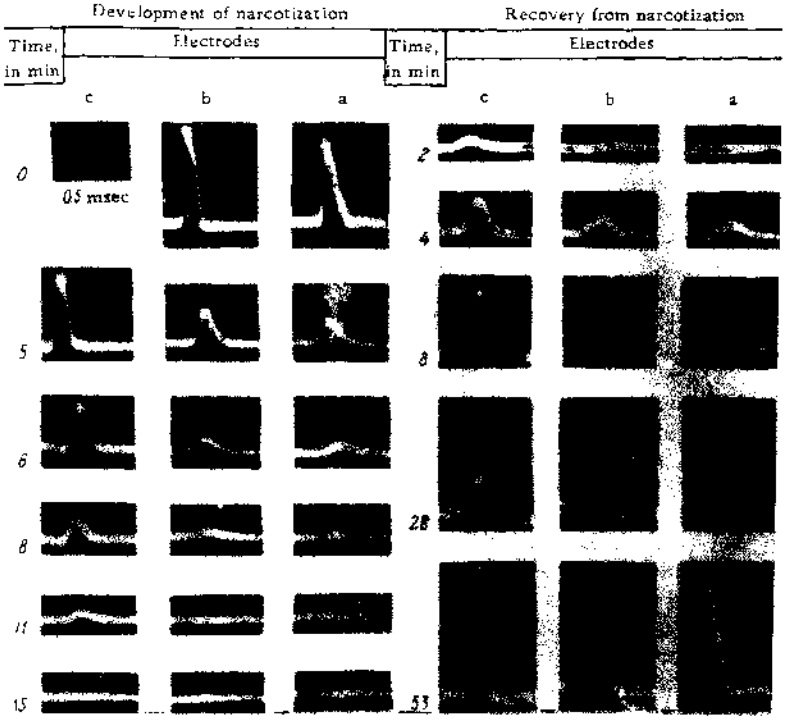


FIGURE 148. Oscillograms taken during stimulation of the nerve from the proximal (c), middle (b), and distal (a) electrodes (according to Nasonov and Rozental', 1952) The photographs were taken at different times after the beginning and end of narcotization.

Subsequently, narcosis was given and 5 minutes later 3 oscillograms (second row) were taken. Here excitability decreased by 20%, the total value of the peak areas decreased considerably and a well-marked decrement appeared (from c to b—30%, from b to a—19%). After 1 more minute, excitability decreased by a further 13%, the area of the peaks also diminished, the decrement increased even more (third row in Figure 148), decrement from c to b equals 40%, from b to a—48%). Later, 8 minutes after beginning of narcotization (fourth row) excitability lessened (48%), decrease of the area of the peaks and increase in decrement (46 and 60%) continued as well. At the distal electrode (a) the peak was hardly discernable, while at the proximal one (c) it was quite well marked. Eleven minutes from beginning of narcosis (fifth row),the peak was clearly seen at the proximal electrode (c) and was missing altogether at the middle (b) and the distal ones (a). Starting from this point (after 10 minutes), all the fibers passed from the first stage of narcosis into the second, and the impulses could spread only with decrement, being therefore limited to a local effect. Finally, after

15 minutes (last row), even the strongest stimuli could not result in appearance of peaks on either of the electrodes.

After that, narcosis was discontinued and the nerve transferred into a moist chamber free of alcohol vapors. As will be seen, in the first row
259 on the right hand half of Figure 148, a small peak appeared 2 minutes after this transfer on the proximal electrode (c) and a barely noticeable one on the middle one (b). On the distal (a) electrode there were no traces of any peak. After 4 minutes, peaks were seen on all three electrodes with a marked decrement (from c to b—42.8%, from b to a—46.6%). Further, the pattern was restored in the reverse order with respect to all indexes, and 53 minutes after cessation of narcosis (fifth row), excitability returned to the initial level (100%), and the decrement practically disappeared (from c to b it equals 6%, from b to a—0%). The areas of the peaks increase 1.5 times in comparison with the initial areas, but for the time being no conclusions can be drawn from this.

Figure 149 shows excitability curves and peak areas at the 3 electrodes during narcotization of a fiber in experiment no. 2 (Table 42). At the beginning, a gradual decrease of excitability is observed corresponding to the first phase of narcosis, and subsequently, when this decrease exceeds 50%, there is a sudden drop of excitability to zero, indicating the beginning of the second phase. Comparison of this curve with curves of the change in peak values shows that while the peak disappears only at the distal electrode, the middle and the proximal peaks are still present. This is the phase when nondecremental conduction disappears, but local spread with decrement still takes place, manifested by the presence of peaks on the 2 proximal electrodes.

When the nerve emerges from narcosis, all these changes occur in the opposite order.

Thus, the appearance on the oscillograms of a clearly expressed decrement with onset of a narcosis, and its disappearance after narcosis, are beyond doubt. It has already been mentioned that decrement was observed in all experiments performed by us (see Table 42). However, in order to prove its real existence it is necessary to disprove those objections which were raised by various physiologists. There are 2 main objections. The first is that a diffusion gradient of the narcotic along the nerve should
260 always exist at the border of the chamber used for narcosis, which may result in the decrement of conduction observed in the experiment. Total narcotization of the nerve, which is possible only on oscillographic recording of the decrement, entirely excludes this objection in our experiments.

The second objection is based on statistical considerations. It is assumed that the nerve fiber is not homogeneous throughout its entire length in relation to resistance to narcosis, and that with sufficiently deep narcosis, different sectors may stop conducting impulses, while intermediate segments still conduct them. In a segment of a whole nerve consisting of many fibers, the probability of the existence of such blocked segments will be greater, the longer the segment. Therefore, on passage through a short nerve segment the overall impulse will drop by a smaller value than on passage through a long segment. This supposedly causes the wrong impression of the existence of decrement in the conduction of impulses by fibers. This apparent decrement will be caused only for statistical reasons, depending on the fact that the longer the nerve segment, the greater the number of fibers which will go out of action.

Table 42

Conduction of Impulse with Decrement in a Narcotized Nerve
(according to Nasonov and Rozental', 1952)

No. of experiment ①	Time ②	Excitability (% of initial) at the distal electrode ③	④ Areas of peaks (% of initial area): c, proximal ⑤	b, middle ⑥	a, distal ⑦	⑧ Decrement (%) from c to b ⑨	from b to a ⑩
1	Before narcosis	100.0	100.0	99.5	96.4	0.50	3.2
	Following cessation of narcotization, after:						
	5 min	64.0	36.0	33.5	23.2	7.0	30.8
	7 "	70.4	57.2	49.4	38.1	13.5	22.87
2	Before narcosis	100.0	—	100.0	95.0	—	5.0
	Following the beginning of narcotization, after:						
	5 min	79.9	66.4	46.9	38.0	29.4	19.0
	6 "	66.6	51.1	30.7	16.0	40.0	47.6
	8 "	48.0	27.0	14.6	5.8	46.0	60.0
	11 "	0.0	21.9	0.0	0.0	100.0	—
	11.5 "	0.0	16.8	0.0	0.0	100.0	—
	13 "	0.0	14.6	0.0	0.0	100.0	—
	Following cessation of narcotization, after:						
	2 min	0.0	8.75	1.46	0.0	83.5	100.0
	4 "	37.12	54.8	31.5	16.9	42.5	46.4
	8 "	54.5	102.2	80.3	60.0	21.5	25.4
	28 "	85.7	135.0	113.1	88.0	16.3	22.2
	53 "	100.0	159.0	150.0	150.0	5.7	0.0
3	Before narcosis	100.0	100.0	107.3	97.5	— 7.3	9.2
	Following cessation of narcotization, after:						
	6 min	54.7	50.0	27.0	15.0	46.0	44.5
	8 "	60.2	70.1	42.6	27.0	39.3	36.7
	10 "	66.9	80.0	60.0	35.0	25.0	41.7
4	Before narcosis	100.0	100.0	108.9	105.7	— 8.9	3.0
	Following cessation of narcotization, after:						
	6 min	64.3	67.4	65.2	62.5	3.3	4.2
	7 "	69.2	89.4	88.9	77.1	0.56	13.3
	9 "	75.0	127.7	127.7	112.3	0.0	12.3
	19 "	100.0	132.1	136.5	136.1	— 3.3	0.3
5	Before narcosis	100.0	100.0	102.1	108.8	— 2.1	— 6.5
	Following cessation of narcotization, after:						
	2 min	65.0	15.5	14.25	19.7	8.1	32.0
	6 "	65.0	44.3	40.2	25.4	9.3	36.9
	7 "	76.7	67.5	61.6	46.5	8.7	24.6
	14 "	108.1	124.0	133.0	134.0	— 7.2	— 0.75
6	Before narcosis	100.0	100.0	107.9	106.1	— 7.9	1.7
	Following cessation of narcotization, after:						
	2.5 min	45.5	70.9	63.0	40.0	11.2	36.5
	3.5 "	50.0	104.8	87.7	57.0	16.4	35.0
	5.5 "	55.5	120.0	98.5	94.0	18.0	9.6
	8.5 "	62.5	132.1	129.2	118.5	2.3	8.3
	11.5 "	100.0	131.0	126.0	129.1	3.9	— 2.5

①	②	③	④			⑧	
			⑤	⑥	⑦	⑨	⑩
7	Before narcosis	100.0	100.0	96.8	103.4	3.2	– 6.5
	Following cessation of narcotization, after:						
	1.5 min	30.0	82.6	63.8	39.3	22.9	38.4
	2.5 "	33.3	120.5	110.6	89.8	8.3	18.8
	3.5 "	43.0	157.5	163.5	142.5	– 4.0	13.1
8	Before narcosis	100.0	100.0	93.6	93.6	6.4	0.0
	Following cessation of narcotization, after:						
	3 min	50.0	56.0	32.3	19.35	42.3	40.1
	4 "	55.5	59.2	49.5	24.65	16.4	50.3
	5 "	62.5	68.7	60.2	40.9	12.4	32.1
	7 "	66.5	88.1	76.4	48.4	13.3	36.7
	10 "	71.5	78.5	78.5	64.5	0.0	17.8
	15 "	83.6	89.2	93.5	65.5	– 4.8	30.0
9	Before narcosis	100.0	100.0	100.0	105.6	0.0	– 5.6
	Following cessation of narcotization, after:						
	5 min	43.0	64.0	53.3	30.3	16.8	43.1
10	Before narcosis	100.0	100.0	104.1	96.0	– 4.1	7.8
	Following cessation of narcotization, after:						
	2 min	30.4	37.5	12.5	10.81	66.6	13.5
	3 "	50.0	58.3	35.0	25.8	40.0	26.3
	4 "	57.3	83.4	75.0	41.6	10.0	44.5
	5 "	57.3	92.5	79.0	65.0	14.6	17.7
	6 "	66.8	91.7	87.5	87.5	4.5	0
	12 "	80.0	98.5	96.0	96.8	2.6	– 0.8
11	Before narcosis	100.0	100.0	97.3	97.3	2.7	0.0
	Following cessation of narcotization, after:						
	1 min	60.0	54.7	53.8	38.7	1.6	28.1
	4 "	60.0	56.5	56.5	43.4	0.0	23.2
	12 "	60.0	50.1	48.1	44.4	4.0	7.7
12	Before narcosis	100.0	100.0	103.0	100.0	– 3.0	3.0
	Following cessation of narcotization, after:						
	6 min	45.6	35.4	26.3	15.4	25.7	41.5
	6.5 "	57.2	41.5	36.9	21.5	11.1	41.8
	7 "	57.2	77.0	63.1	41.5	18.0	34.3
	7.5 "	62.3	87.7	80.0	57.0	8.8	28.8
	8.5 "	68.5	101.5	98.5	57.0	3.0	42.3
	9.5 "	68.5	109.0	104.5	46.2	4.1	55.8
	10.5 "	76.0	152.3	118.4	106.0	22.3	10.5
	12.5 "	76.0	133.8	127.5	112.5	4.7	11.8
	16.5 "	85.5	143.0	141.5	126.2	1.1	10.8
	20.5 "	85.5	137.0	137.0	131.0	0.0	4.4
13	Before narcosis	100.0	100.0	99.2	91.3	0.8	8.0
	Following cessation of narcotization, after:						
	8 min	—	14.8	13.9	11.3	6.0	18.7
	12 "	25.0	24.4	18.25	13.2	25.3	27.7
	14 "	44.5	18.25	18.25	13.2	0.0	27.7
	17 "	50.0	16.5	14.8	12.2	10.7	17.6

①	②	③	④			⑧	
			⑤	⑥	⑦	⑨	⑩
14	Before narcosis	100.0	100.0	101.2	99.0	— 1.2	2.2
	Following the beginning of narcotization, after:						
	11 min	38.5	72.5	65.0	52.2	10.3	19.7
	19 "	38.5	63.6	57.4	47.0	9.8	18.1
	Following cessation of narcotization, after:						
	1.5 min	16.65	14.7	10.2	2.58	30.6	74.7
	3 "	20.8	59.3	49.0	26.5	17.4	45.9
	4 "	22.7	61.2	53.0	35.0	13.4	34.0
	5 "	27.8	69.0	62.5	37.0	9.4	40.8
	6 "	31.3	85.4	70.0	47.0	18.0	32.9
	12 "	50.0	75.6	63.7	61.8	15.8	3.0
	20 "	73.0	51.0	42.0	44.6	17.7	– 6.1
	30 "	100.0	44.0	37.0	38.2	14.5	– 1.6

Notes: 1. In experiment no. 14, 10% alcohol caused complete narcosis only after repeated narcotization.
2. The minus sign designates increment (or increase of the area).

This objection is refuted by data cited by Bullock and Turner (1950) in their studies on single fibers. Figure 127 shows that at a certain stage of narcosis the amplitude of the peaks on the proximal pair of electrodes (I) is considerably larger than on the distal one (II). At a still deeper narcosis, the proximal electrodes record only a monophasic peak, while the distal electrodes show no peak at all. Decrement is obvious here. It is self-evident that statistical considerations relating to a nerve consisting of a large number of fibers are not applicable here.

We have earlier given a short review of the literature on conduction of impulses by a narcotized nerve segment. We shall now attempt to show how these contradictory data relate to the facts and conclusions arrived at by us.

In early papers on this question numerous authors concluded, on the basis of indirect data, that during narcosis impulses spread with decrement. Lucas described this decrement in the form of a straight line (Figure 150,A) 261 and Verworn described it in the form of a curvilinear relationship (Figure 150, B). According to the latter author, if the impulse is higher than the threshold of the healthy nerve on reaching the end of the chamber, it immediately jumps to the initial value. In the opposite case, the impulse does not leave the chamber. There is no doubt now that all these authors dealt with the second phase of narcosis, when the majority of fibers started to conduct impulse with decrement. In this case the limit length of the narcotized nerve segment should depend on the time or on the depth of narcosis. The first phase of narcosis in which there is only a decrease in the level of the impulse could have been overlooked by the authors, since in the majority of cases they judged the decrement by contraction of the muscle.

The study of dependence of the time of narcotization on the length of the chamber, associated with the problem of decrement, has led to the establishment of the rule expressed by the curves given in Figure 141. It has been shown above that curves of this type may theoretically be deduced on the basis of our theory of "gradual" excitation. From the course of these curves it will be seen that the time of narcosis depends on the length of the narcotized nerve segment, which is well expressed in short segments and

becomes very small and practically indiscernible in long ones. According to the data of P.O. Makarov this dependence may be seen in segments up to 10 mm long but not in longer ones.

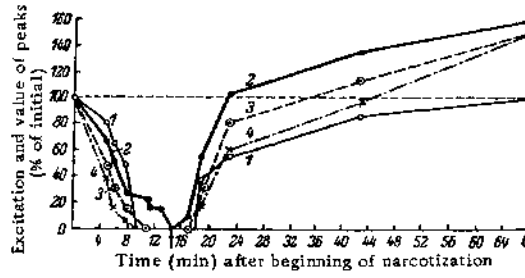


FIGURE 149. Change in excitability of (1) the nerve and of the value of peaks from the proximal (2), middle (3) and distal (4) electrodes on entry into, and exit of the nerve from narcosis (according to Nasonov and Rozental', 1952).
The arrow indicates termination of narcosis.

Kato (1924) also observed (purely empirically), that in chambers not longer than 6 mm the dependence of time of narcosis on the length of the narcotized nerve segment is well seen, while in longer chambers there is no such relationship. He explained this fact by the existence of a diffusion gradient of the narcotic drug and he began to work exclusively with large chambers, i.e., he created conditions under which it is practically impossible to prove the existence of decrement in the second phase of narcosis, using a nerve muscle preparation from a frog. Under such experimental conditions Kato could observe only the first phase of nondecremental conduction, after which, in his opinion, conduction stops abruptly.

In reality, however, there is conduction with decrement in this phase, and it can be observed either with short sectors of a narcotized nerve, or recording the decrement, not by the muscular reaction but directly by oscillography. As already mentioned, Kato's statement that the decrement in conduction during narcosis is an artifact, caused by diffusion of the narcotic, is ill-founded.

Makarov (1939) found a dependent relationship between the time of narcosis and the length of the segment, during narcosis caused by mechanic-
252 al pressure (Figure 141, D), which entirely excludes diffusion. We obtained an obvious decrement under conditions which also excluded diffusion of the narcotic. This erroneous explanation of decrement is the source of Kato's main error which led him to the theory of conduction during narcosis (Figure 150, C). This is essentially correct, but reflects only the first phase of narcosis.

As compared to Kato, the theory of Davis, Forbes, Brunswick and Hopkins (Figure 150, D) is a step forward. These authors recorded the decrement on a string galvanometer. They obtained a fine picture of the 2 phases of narcosis very close to that given by us (Figure 150, E). However, they considered the second phase to be an artifact, on the basis of the

statistical objections mentioned above. The inconsistency of these objections was shown by our data. These authors deny the fact of diffusion of the narcotic, and they are therefore forced to compromise and recognize both decrement of conduction at the beginning of the narcotized segment, and increment at its end (Figure 150, D). In this respect they approach one step closer to the correct solution of the problem but only give a half-way solution insofar as, together with Kato, they do not recognize the existence of decrement in the second phase of narcosis.

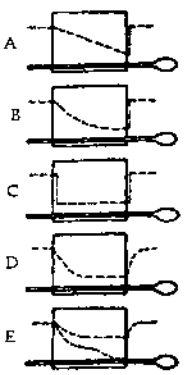


FIGURE 150. Scheme illustrating the views of various physiologists on the passage of impulses through a narcotized nerve segment (according to Nasonov and Rozental',1952)

A—Lucas (1913, 1917); B—Verworn (1914); C—Kato (1924, 1934); D—Davis, Forbes, Brunswick and Hopkins (1926); E—Nasonov and Rozental',(1952).

Thus, our data on the conduction of impulse by a narcotized nerve, strictly speaking, are not opposed either by the earlier authors who agreed to the existence of decrement, or by the more recent ones, who did not accept its existence. Both groups described only one aspect of the phenomenon. The earlier writers, using indirect methods, could not detect the first phase in their work with the whole nerve, and detected only a decrement. Kato and his followers worked with a single fiber, but arbitrarily refusing to work with short segments, they missed the second phase which they erroneously considered as a sudden and complete loss of conductivity.

Spontaneous Rhythmic Activity of Conducting Fibers

One of the conclusions following from our considerations is that the so-called spontaneous rhythmic activity of the conducting fibers should invariably occur when the threshold of electric excitability of the nerve falls below a certain level. From this it follows that if any agent causes such activity in the nerve or muscle, this condition should always be attained through increase in initial excitability.

Certain data confirming this hypothesis may be found in the literature. However, while there is an extensive literature on the origin of rhythm in nerves and muscles influenced by various agents, and also on experimental increase in excitability, there are few studies showing the direct connection between the two. In some of the latter studies it is hinted at that the appearance of a rhythm is necessarily accompanied by increased excitability, but no direct proof is given. In other words, there are indirect indications of both increase in excitability and automatic activity. In Vvedenskii's monograph "Excitation, Inhibition and Narcosis" published in 1901, the connection is mentioned (page 71-78) between increased excitability and onset of rhythmic activity of the nerve during restoration after
263 narcosis. The work of Subbotin (1866), Gabrichevskii (1888), Saint-Hilaire (1890), Blumenthal (1896), Loeb (1901, 1910), Gulinova (1906), Mayer (1911), Rezvyakov (1914-1915) and also those of Kvasov and Naumenko (1936) and Indzhikyan (1937), should be mentioned. In the majority of these studies

the excitability thresholds were determined by electric induction shocks from a DuBois-Reymond coil.

Data in the literature describing the appearance of a rhythm following the action of a constant local stimulus also confirm that increase in excitability causes this phenomenon. Thus, Vvedenskii (1883) caused a weak tetanus by stimulation through a nerve treated with a concentrated solution of NaCl. This tetanus was strengthened by the action of direct current. Slight drying acted similarly to hypertonic solutions. Momsen, Grunhaagen and Biederman (1895), cited by Kvasov, 1937) found that alteration of the nerve by hypertonic solutions, which in itself does not cause these rhythms, nevertheless facilitated the occurrence of a multiple response of the nerve on closing and opening of direct current.

These authors found that in the vicinity of a cross-cut a closing and opening tetanus is facilitated. According to this, Yudenich (1928) has shown that in the vicinity of a cross-cut of the nerve an increase in excitability is observed.

On the other hand, calcium ions, which are known to lower nerve excitability, interfere with the multiple response to direct current (Solandt, 1936a, 1936b, Katz, 1936).

Weak stimuli cause an increase in excitability. In this respect the observations of Kvasov and Ushinskaya (1948), who obtained a multiple discharge in muscle fibers by the action of weak pricking, are very interesting. This effect was obtained only when excitability of the muscle was previously increased by the action of glycerol or by drying.

In general, the vast majority of facts at our disposal indicate that the rhythmic activity of nerve and muscle fibers is closely associated with increase in excitability of the latter. As already mentioned, the investigators originally showed a tendency to look for a causal relationship between these two phenomena. Recently, however, after the appearance of papers by Hill and his students Solandt, Katz and others (Hill, 1936; Hill, Katz and Solandt, 1936; Solandt, 1936a, Katz, 1936), the appearance of rhythmic activity in tissues was more frequently associated with decrease in the so-called accomodation ability. At first, this property was considered as an adaptive reaction of the fibers to the action of the excitant, and it was considered that if this adaptability to the excitant was marked, the fiber may give one reaction, at best. If this adaptability was small and the protoplasm was not able to adapt to the action of the excitant, impulses arose during a long time period and rhythmic activity occurred. Quite recently serious doubts appeared as to the adaptive nature of this phenomenon which was called "accomodation". This was demonstrated quite convincingly by Averbakh (1948) and by Khodorov (1950a-1950d), who again confirmed the neglected but nonetheless correct views of the Russian physiologist Verigo (1888), concerning the nature of physiological electrotonus. From this point of view, increase in excitability threshold on gradual increase of excitatory current (which is wrongly called accomodation) is a gradual increase in cathodic depression, and has nothing in common with adaptation.
264 This question will be discussed in detail in a later chapter.

If, according to the data of the British authors (Solandt, 1936a, 1936b; Katz, 1936), decrease in speed of "accommodation" in certain cases causes rhythmic activity of the fiber, this happens only because this is frequently (although not always) accompanied by increased excitability. Khodorov (1950) noted an exception to this rule, i.e., calcium chloride always lowers

excitability and suppresses the rhythm; at the same time, it affects the speed of "accomodation" in a biphasic fashion. At first, for a number of hours, it causes a marked decrease in it (almost twofold) and subsequently, in the maximal stage of its action, it increases it*.

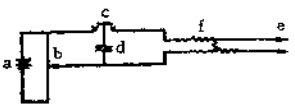


FIGURE 151. Apparatus (according to Nasonov and Suzdal'skaya, 1954)

a—battery; b—rheostat; c—metronome; d—condensor; e—calomel electrodes; f-Lapique's shunt.

These diverse data from the literature forced us to test the facts in order to ascertain whether the theoretical concepts were valid or not. It was necessary to ascertain whether agents causing nerve fiber rhythms necessarily led it to this stage through a stage of increased excitability (Nasonov and Suzdal'skaya, 1954).

To avoid complications due to possible effect of the agents on the ability of the nerve to reproduce the rhythm, we used as stimulus not induction shocks but discharges of a condenser of 16 microfarads, built according to the diagram in Figure 151.

The experiments were performed in the winter of 1949/50 in the following manner. The middle part of the nerve from the nerve-muscle apparatus of the frog, 2.5-3 cm long, was immersed in the investigated solution (Figure 152). At short time-intervals the nerve was taken out and its treated part placed on the wires of the calomel electrodes, fixed on glass at a constant distance (5 mm) from each other. The nerve was stimulated by discharges of the condenser (d), closed by the metronome (c) once per second (Figure 151). The threshold was determined by displacement of the lever of the rheostat (b), due to which the value of the input voltage was proportional to the values
265 found. During estimation of the thresholds, which lasted 2-3 minutes, the nerve was kept in a moist chamber.

The study of the action of a number of agents on the frog nerve (drying, NaCl, sodium oxalate, glycerol, KCl, CsCl, ethyl alcohol, $CaCl_2$), using the above method, has shown that in all cases where rhythmic activity appeared in the nerve, it was indeed preceded by increase in excitability.

In order to check this we, together with Averbakh, performed experiments in which nerve excitability was determined more accurately by the oscillographic method (Nasonov and Averbakh, 1951). In addition, we could also check another corollary to our theory, namely that the rhythm of automatic activity is determined by the duration of the entire cycle of excitation. A new wave of excitation arises immediately after that, as soon as the fiber returns to the resting condition after a preceding excitation.

Let us imagine that a nerve consisting of many fibers entered a state of rhythmic activity, whereupon all the fibers worked asynchronously, in a haphazard manner. If a sufficiently strong stimulation is applied to such a nerve by means of electric current, the rhythm of all fibers should become synchronized at least for a certain time. The electric shock may find the asynchronously acting fibers in a state of absolute or relative refractivity. Since this shock is sufficiently strong, in all the fibers with relative

---

* Simple increase in speed of accomodation by the action of $CaCl_2$, which has been described by Solandt (1936a), was obtained only in frogs previously kept in the cold. According to Khodorov, in frogs kept in warm temperatures these changes were always biphasic.

refractivity excitation will arise simultaneously, while the fibers in the absolute refractory period are anyway already in the initial stage of the excitation cycle. In other words, an external stimulus will lead all the fibers to the beginning of the excitation cycle and will force them, so to speak, to start simultaneously. If the duration of the excitation waves of the fibers composing the nerve does not vary very much, the following burst of excitation should set in more or less simultaneously in all the fibers, since the time of its appearance will be determined by the completed excitation cycle. Such a simultaneous appearance of excitation may also be expected further on, but each time the synchronizing of the work of the fibers should be more and more disturbed, as a result of imperfect coincidence of duration of the waves in different fibers.

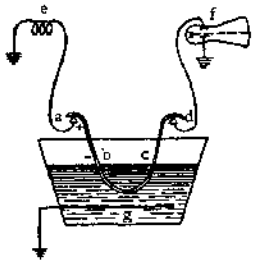


FIGURE 152. Apparatus for stimulation and oscillographic recording of altered nerve (according to Nasonov and Averbakh, 1951) For explanations see text.

Thus, the aim of these studies was to check experimentally the three following postulates of the theory:

1) Transition of the nerve fiber to rhythmic activity should be accompanied by increase in excitability.

2) The duration of the wave of rhythmically working fibers should approximately correspond to the overall duration of the absolute and relative refractory periods.

3) Electric stimulation of a rhythmic nerve should for some time synchronize the rhythmics of the separate fibers entering in the composition of this nerve.

In all experiments, sciatic nerves of frogs, isolated from the spinal cord up to the knee joint, were used as the object of study. The middle part of the excised nerve was immersed in a vessel with liquid (test solution), while its ends, protruding above the surface of the liquid, were placed on electrodes a and d, as illustrated in Figure 152. The electrode a (anode) served as one of the stimulating electrodes, while the other pole of stimulation (cathode) was grounded. The liquid in the vessel was also grounded under such conditions that the actual site of stimulation was the region of the nerve (b) adjacent to the surface of the liquid. The 266 stimulus used was a series of short, sharply-ending monophasic impulses of one and the same charge.

Electrode d, on which the opposite killed end of the nerve was placed, served as one of the recording electrodes and was connected with the amplifier of the oscillograph (f). The other pole of the oscillograph was grounded. Under such conditions the oscillograph gave a monophasic recording of the waves of excitation passing between c (surface of liquid) and d.

The liquid was grounded by a silver plate (g) fixed to the bottom of the vessel, due to which recording of the current loop was completely eliminated.

For recording of excitation potentials a cathodic oscillograph with a double beam tube and amplifier, the frequency characteristic of which in the interval from 5 to 10,000 cps was rectilinear, was used. The maximal sensitivity of the instrument corresponded to 25 mm per 1 millivolt. On the oscillograph screen the pattern of biocurrents fixed by synchronization of

the rhythm of excitation was noted; the excitation was found to coincide with the beginning of the straight path of the beam, which allowed direct observation of the propagating impulse.

The action of the following solutions, bringing the nerve into the state of automatic activity, was studied: 1%, 2%, and 4% sodium oxalate, 4% and 8% sodium citrate, 3% and 6% sodium chloride, 15% and 30% glycerol. The first two solutions (sodium oxalate and sodium citrate) act primarily as calcium precipitants, while the mechanism of action of hypertonic solutions of NaCl and glycerol is in all probability by way of dehydration of the nerve fiber protoplasm.

All experiments were performed in the following manner. The prepared nerve, after being kept half an hour in Ringer's solution, was placed on the electrode and the investigated liquid poured into the vessel. After that, before the effect of the solution could be observed, the initial threshold of excitability was measured at once and 2 patterns of the oscillograms were taken—with and without excitation. A strong stimulus, 5-10 times greater than the threshold, was always used. Later, this procedure was repeated every two to three minutes until the onset of automatic activity, after which exact determination of the threshold was no longer possible, due to the continuous repeating bursts of spontaneous excitation. Therefore, after the appearance of automatic activity only patterns of the oscillograms, with and without excitation, were taken from time to time.

The results of one experiment with 4% sodium oxalate are illustrated in the data of Table 43 and Figures 153 and 154.

From Table 43 it follows that the threshold of excitability fell continuously from the very first minutes of immersion of the nerve in the solu-
267 tion (see also Figure 153, A). At this time the response of the excited nerve on the oscillogram has the form of a straight line (Figure 154, A), since there is no spontaneous activity. On excitation a peak appeared (Figure 154, B), but the remaining part of the oscillogram represented an even line. At the tenth minute the threshold fell to 22% and spontaneous activity began. Examination of the pattern on the screen shows that prior to that, the straight line started to oscillate along its entire length. No regularly propagated waves were seen.

An instantaneous photograph also does not give any regular pattern of waves (Figure 154, C), and there is only a series of wavy lines obtained because each fiber of the nerve shows a rhythm quite independent of the other fibers. However, the picture changed at once, as soon as the nerve was stimulated. Figure 154, B, shows that in such a case, in addition to the large peak resulting from direct action of the electric stimulus, a second peak appeared some distance from the former (in the given case corresponding to 7 milliseconds), of somewhat smaller dimensions. On an oscillogram with a smaller speed of unfolding (Figure 154, E) a third and a fourth peak occur after the second peak at similar intervals. However, if the stimulus was eliminated, these additional peaks disappeared at once and again a disorderly undulating line was seen (Figure 154, C).

It has already been said above that these phenomena are postulated by our theory and are explained by the synchronizing effect of a strong stimulus.

The oscillograms show that the second peak is considerably smaller in height and in area than the first. This is explained by the fact that at the beginning, not all the nerve fibers enter rhythmic movements spontaneously.

Only the most sensitive among them do so. After being kept for a longer time in oxalate the nerve showed second peaks of increasing magnitude, due to new fibers entering into spontaneous activity, the time interval between the peaks being somewhat shortened (In Table 43 the duration of the waves shortened from 7.5 to 6.5 milliseconds). Further on the third and fourth waves became more diffuse. This dissociation took place due to an unequal duration of the waves in the different fibers. At the same time, the mere fact of appearance of the second, third and fourth peaks indicates that the duration of the waves in different fibers did not vary greatly.

Table 43

Appearance of rhythmic activity in frog nerve under influence of 4% sodium oxalate (according to Nasonov and Averbakh, 1951)

Nerve kept in solution for duration of (min):	Threshold of excitability (% of initial)	Duration of wave of rhythmic activity (milliseconds)	Rhythmic activity
0	100	—	—
2	78	—	—
4	50	—	—
6	39	—	—
8	28	—	—
10	22	7.5	+
12	—	7.0	+
16	—	6.5	+

Table 44 summarizes the data on the action of sodium oxalate on the frog nerve. It follows from these that, on the average, when the threshold of excitability fell to 37% of the initial rate, automatic activity appeared after 12 minutes. The mean duration of the wave of rhythmic activity was 8 milliseconds at room temperature (20° C).

The effect of sodium citrate is very similar to that of oxalate. Table 45 and Figure 153, B indicate that here, too, the threshold of excitability 268 begins to drop at once. Rhythm activity in the nerve appeared when the threshold fell to 45% of the initial level. The wave length of rhythmic activity corresponded to 11 milliseconds at the beginning, decreasing to 9 milliseconds after 1 hour.

Figure 155 shows oscillograms of another nerve subjected to the action of 4% sodium citrate. A powerful peak occurs resulting from direct excitation; alongside it, a second peak is barely noticeable (Figure 155, A). Together with the first peak a second and third are seen, the second peak being somewhat smaller in area than the first (Figure 155, C).

Table 46 summarizes the data on the effect of 4% sodium citrate on the frog nerve. The average figures are very close to the results obtained after the action of 4% sodium oxalate (Table 44).

That is the situation with effect on the nerve of substances precipitating calcium from solution. Rhythmic activity caused by dehyrating solutions will now be considered. Among them 3% and 6% NaCl and 15% and 30% glycerol were studied. All these solutions cause spontaneous activity in the nerve after a certain interval.

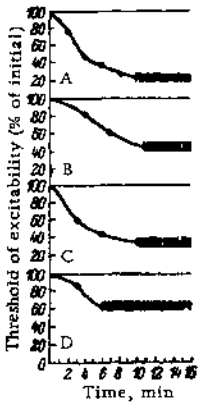


FIGURE 153. Drop in the excitability threshold of a frog nerve, in solutions inducing in the nerve
270 a state of automatic activity (according to Nasonov and Averbakh, 1951)

A—4% sodium oxalate; B—4% sodium citrate; C—6% sodium chloride; D—30% glycerol. The vertically shaded columns designate rhythmic activity.

As shown in Table 47, the excitability of a nerve placed in a 6% NaCl solution started to increase at once, and at the instant the threshold fell to 35% of the initial value, rhythmic activity appeared (Figure 153, C). Here, as in the previous cases, undulation along the course of a straight line and rapid propagation of separate disorderly waves (Figure 156, A) were seen on the oscillograph screen. Application of a stimulus synchronized the activity of the fibers, but less so with the action of oxalate and citrate. Figure 156, C shows clearly only one additional wave arising as a result of synchronization of rhythmic activity, and there are only traces of the subsequent waves. However, this second wave is quite clearly seen, not only on strong stimulation, but with a slightly above-threshold stimulus (Figure 156, B). It is obvious that after the action of dehydrating agents the duration of the wave in different fibers varied much more than on the action of agents precipitating calcium.

Table 48 shows data on the effect of 6% NaCl. Comparing the average values in Table 48 with those in Tables 44 and 46, attention is drawn to the fact that in the given case there are no basic differences between this effect and that of oxalate and citrate with regard to the threshold at which rhythmic activity sets in (37, 42 and 48%), and the time necessary for the onset of the latter (11.7, 11.3 and 8.7 minutes). As far as the duration of the wave of automatic activity is concerned, it is much shorter in the case of 6% NaCl (4.7 milliseconds, instead of 8.1 and 8.7 milliseconds).

The effect of 15% and 30% glycerol is very similar to that of 6% NaCl. Table 49 and Figure 153, D show that here, too, immediately after immersion of the nerve in the solution, its excitability begins to increase, and when the threshold falls to 63% of the initial value, spontaneous rhythmic activity begins, which is synchronized upon excitation. In the given case, as with the action of 6% NaCl, only a second wave may be observed, which in its duration (4 milliseconds) approaches the wave caused by sodium chloride.

271 The aim of the above-described experiments was to check by experiment the results postulated by the theory of "gradual" conduction of a stimulus. Let us see to what extent we succeeded in doing so.

The first of the three conclusions was the statement that the transition of any nerve to a state of automatic activity should be accompanied by an increase in excitability. This assumption has been completely confirmed in the case of all four agents studied. In our opinion, for each nerve there should exist a level of such excitability that on exceeding it, rhythmic activity should automatically occur. In this sense the term "threshold of automatic activity" may be used.

Elsewhere the question will be discussed of what may determine the value of the threshold of automatic activity. Here we shall only give the

mean values of these thresholds obtained under the given conditions for different agents. They do not greatly differ from each other; (for oxalate—37% of the initial threshold, citrate—42% and NaCl—48%).

Table 44

Effect of 4% sodium oxalate: Summary of data. (according to Nasonov and Averbakh, 1951)

Serial number	Excitability threshold at the moment of appearance of rhythmic activity (% of initial)	Duration of wave of rhythmic activity (milliseconds)	Time passed before onset of rhythmic activity (min)
1	58	7.0	4
2	37	8.0	12
3	27	9.5	18
4	22	7.5	10
5	50	9.0	8
6	30	7.5	18
Arithmetic mean	37	8.1	11.7

Table 45

Appearance of rhythmic activity in frog nerve under the influence of 4% sodium citrate (according to Nasonov and Averbakh, 1951)

Nerve kept in solution for duration of (min):	Threshold of excitability (% of initial)	Duration of wave of rhythmic activity (milliseconds)	Rhythmic activity
1	100	—	—
4	84	—	—
7	63	—	—
11	45	11	+
21	—	10.5	+
31	—	10.0	+
31	—	9.5	+
61	—	9.0	+

According to the second conclusion, the duration of the waves of rhythmically active fibers should approximately correspond to the sum of durations of the absolute and relative refractory phases.

In addition to what has already been mentioned in this respect, it may be added that an agent causing increase in excitability may affect, in one or other way, the duration of the phases. Therefore only an approximate, but not an absolute correspondence may be described. The duration of absolute refractory period of a frog nerve is 2-3 milliseconds. The relative refractory phase exceeds the absolute 3-4 times, and consequently, the normal cycle of excitation in the frog nerve lasts approximately 8-15 milliseconds.

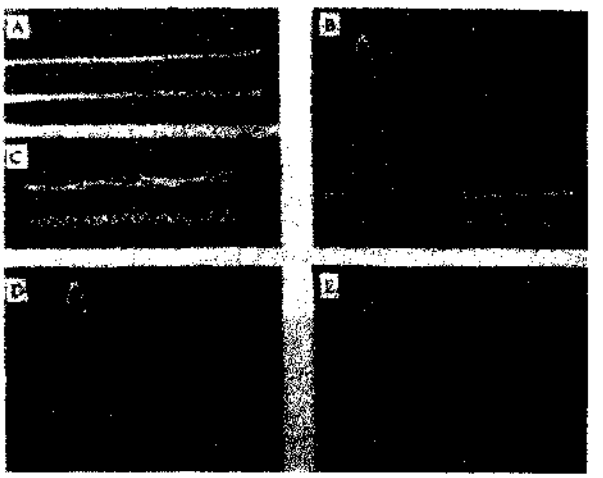


FIGURE 154. Oscillograms of a frog nerve treated with 4% sodium oxalate (according to Nasonov and Averbakh, 1951)

A—the nerve before beginning of rhythmic activity; B—same nerve subjected to excitation; a well-expressed peak is seen; C—same nerve after 16 minutes in a sodium oxalate solution; disorderly rhythm of the different fibers is seen; D—same nerve on excitation; in addition to the first peak, a second peak is clearly seen, formed as a result of synchronization of rhythmic activity of different fibers; E—same picture at a lower speed of unfolding; in addition to the first peak, a second, third and fourth peaks are seen.

At room temperature the duration of the wave of automatic activity in the case of oxalate was 8.1 milliseconds and for citrate—8.7 milliseconds, i.e., values well agreeing with the expected ones were obtained. In the case of dehydrating agents a somewhat shorter duration was observed (4-5 milliseconds). It is highly probable that these agents somewhat shortened the duration of the refractory periods.

272 Thus, this second requirement of the theory has also been experimentally confirmed.

Finally, the third consequence subjected to experimental examination was that the electric excitation of a nerve active with a disordered rhythm should synchronize the rhythmic activity of the separate fibers entering in its composition. As we have seen, this requirement of the theory was also confirmed experimentally in all cases.

According to our theory, the rhythm of the automatically working fiber is determined by the duration of the entire excitation cycle. As soon as this cycle was completed, the fiber was again involved in the excitation effect, since a rhythmically active fiber is in an unstable condition and the slightest excitation originating at any point on it will inadvertently increase

to the maximal possible. However, there may be another mechanism of rhythmic activity, an exogenous one, where the rhythmically arising excitation is caused by certain rhythmic processes not related to the excitation cycle. Such a rhythmic excitation could occur, for instance, in certain cyclic processes of intracellular metabolism (Karasik, 1947), or in impulses coming from other regions of the organism. It is obvious that in these cases, the excitation should not synchronize the disorderly rhythmic activity of the different fibers. Our experiments thus negate this second explanation, at least with regard to rhythmically active frog nerve.

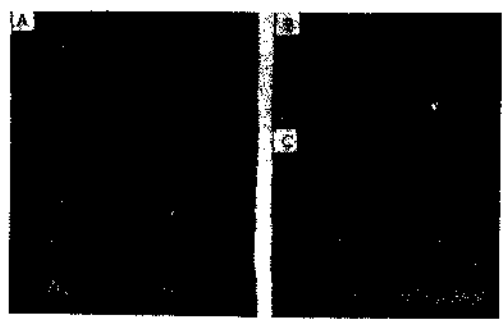


FIGURE 155. Oscillograms of a frog nerve treated with 4% sodium citrate (according to Nasonov and Averbakh, 1951)

A—excitation after keeping the nerve 7 minutes in the solution; in addition to the main peak, there is a second one, caused by synchronization of the rhythmic activity; B—same nerve after 6 hours; picture taken without excitation; disorderly rhythmic activity of the fibers occurs; C—same nerve upon excitation; 2 additional peaks appear as a result of synchronization of rhythmic activity.

Similar phenomena associated with the appearance of rhythmic activity were also observed by us on single nerve fibers of a crab (Hyas araneus) (Nasonov, Averbakh and Komarova, 1950).

The relation of increased excitability to automatic activity in striated muscle fibers has been studied recently by Dzhamusova and Ponomarenko (1954), who experimented with the sartorius muscle of a grass frog and the retractor muscle of a worm (Priapulus caudatus, Barents Sea). Excitation was induced by condensor discharges of 30 microfarads through nonpolarizing electrodes (Figure 151). Thus, excitation was determined by the value of the rheobase. The results showed that in the case of muscles, a number
273 of agents (KCl, Na_2SO_4, HCl, sodium citrate, calcium-free Ringer's solution, the dye dahlia-violet, etc.) caused automatic activity through increase in excitability (Figures 157 and 158). It is interesting that this increase passed into rhythmic activity after fall of the threshold of excitability to a

certain level, which did not depend on the initial threshold. This observation agreed completely with our theoretical considerations.

Table 46

Effect of 4% sodium citrate: Summary of data (according to Nasonov and Averbakh, 1951)

Serial number	Excitability threshold at the moment of appearance of rhythmic activity (% of initial)	Duration of wave of rhythmic activity (milliseconds)	Time passed before onset of rhythmic activity (min)
1	45	11	11
2	40	7	10
3	40	8	10
Arithmetic mean	41.7	8.7	10.3

Table 47

Appearance of rhythmic activity in frog nerve under the influence of 6% NaCl (according to Nasonov and Averbakh, 1951)

Nerve kept in solution for duration of (min):	Threshold of excitability (% of initial)	Duration of wave of rhythmic activity (milliseconds)	Rhythmic activity
0	100	—	—
3	60	—	—
6	47	—	—
10	35	5.5	+
15	—	5.5	+

Table 48

Effect of 6% NaCl: Summary of data (according to Nasonov and Averbakh, 1951)

Serial number	Excitability threshold at the moment of appearance of rhythmic activity (% of initial)	Duration of wave of rhythmic activity (milliseconds)	Time passed before onset of rhythmic activity (min)
1	35	5.5	10
2	47	4.2	10
3	61	4.5	6
Arithmetic mean	47.7	4.7	8.7

Table 49

Appearance of rhythmic activity in frog nerve under the influence of 30% glycerol (according to Nasonov and Averbakh, 1951)

Nerve kept in solution for duration of (min):	Threshold of excitability (% of initial)	Duration of wave of rhythmic activity (milliseconds)	Rhythmic activity
0	100	—	—
3	88	—	—
6	63	4	+
15	—	4	+

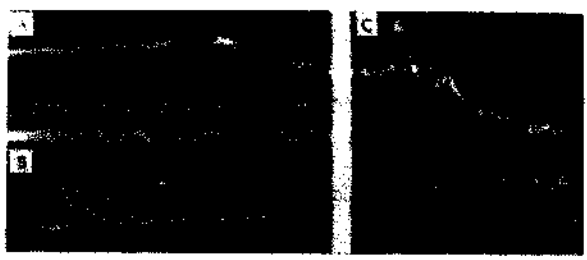


FIGURE 156. Oscillogram of frog nerve treated with 6% NaCl (according to Nasonov and Averbakh, 1951)

A—a nerve showing rhythmic activity without excitation, 10 minutes after immersion in the solution; B—same nerve subjected to threshold stimulus; in addition to the main peak another peak occurs as the result of synchronization of rhythmic activity; C—same nerve upon maximal excitation.

In the experiments of Dzhamusova and Ponomarenko a solution of NaCl was shown to act as an exception to the rule of transition to rhythmic activity through increased excitability. This agent caused rhythmic activity in concentrations above 1.25%, against a background of developing contraction, but the excitability of the muscles decreased rather than increased. A suggestion was made that not the muscle, but the nerve dendrites passing into the nerve fibers are the first to start rhythmic activity. In order to check this, sartorius frog muscles were denervated and taken out for experiments after 34-38 days. Results showed that a 5% solution of NaCl caused only contraction of the denervated muscles (Figure 159, A), while its innervated twin muscle showed a well-expressed rhythmic activity (Figure 159, B). One would have thought that the denervated muscle was incapable of rhythmic activity, but after the action of 1% solution of sodium citrate, both normal and denervated muscles showed rhythmic activity (Figure 160).

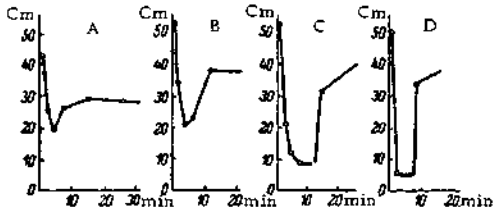


FIGURE 157. Change in excitability threshold of sartorius frog muscle, in solutions of sodium citrate (according to Dhzamusova and Ponomarenko, 1954)

Concentration of solution: A—0.12%; B, C—0.25%; D—0.5%. The abscissa shows the time of action of the substance, and the ordinate shows the threshold strength of the current. The shaded segments designate rhythmic contractions of the muscle.

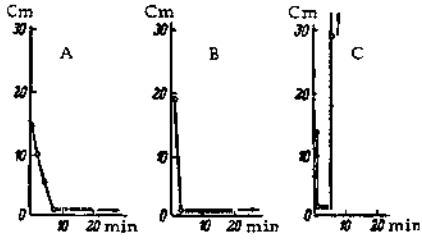


FIGURE 158. Change in excitability threshold of the retractor muscle of Priapulus caudatus in solutions of sodium citrate (according to Dhzamusova and Ponomarenko, 1954)

Concentration of solution: A--2%; B—4%; C—6%. The arrows indicate the direction of the changes. The remaining legend as in Figure 157.

These results show that in the given conditions frog muscle is not capable of rhythmic activity under the effect of strong solutions of NaCl. Secondary rhythmic contractions are observed in this muscle only when viable terminal nerve dendrites passing to the motor plates are preserved. Rhythmic impulses arising in these muscles under the influence of NaCl force the muscle to contract spasmodically. This is a good example of how an apparent exception confirms the rule.

All conclusions with regard to rhythmic, spontaneous activity of nerve elements are especially important and interesting to us because, practically speaking, only rhythmic activity takes place in the organism. Bursts of such rhythmic activity originate in the nerve endings of most different receptors when acted upon by stimuli. Such more or less protracted bursts of rhythmic activity are also sent out from the nerve center in the direction of the periphery. In terms of the theory developed here, transitions to rhythmic activity should occur as a result of increased excitability, when a β-type of excitability passes into a γ-type of excitability (Figure 129).

274

It may be assumed that after the action of stimuli on nerve endings of the receptors, an early stage of parabiosis takes place, characterized by increased excitability. If the excitability of these endings is in itself

sufficiently high, being on the border between β and γ, then after a very slight stimulation causing an increase in excitability, rhythmic activity will appear in these endings, due to the fact that the β type of excitability was transformed into the γ type of excitability. Once it has arisen, such a burst of impulses will move further along the ordinary fiber possessing β type excitability. The muscle fibers in the above-mentioned experiments of Kvasov and Ushinskaya (1948), may serve as a model of a receptor. These authors artificially increased the excitability of fibers by preliminary treatment of the preparation with glycerol, or by drying, after which very weak pricking caused the appearance of multiple impulses.

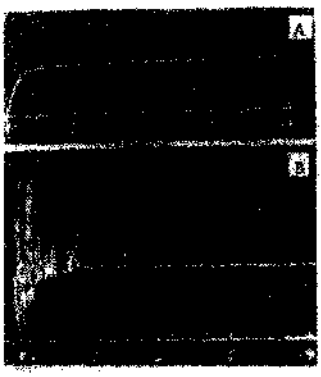


FIGURE 159. Effect of 5% NaCl on (A) denervated and (B) nondenervated sartorius frog muscle (according to Dzhamusova and Ponomarenko, 1954)
The horizontal line shows time (minutes). The arrow designates beginning of action of the agent.

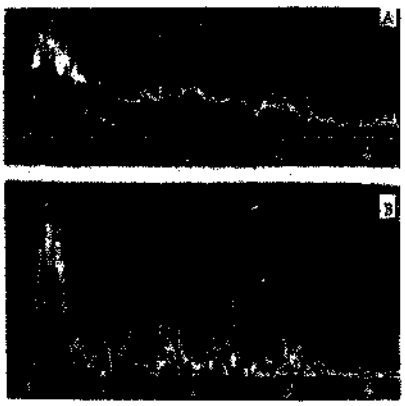


FIGURE 160. Effect of 1% sodium citrate on (A) denervated and (B) nondenervated sartorius frog muscles (according to Dzhamusova and Ponomarenko, 1954)
Legend as in Figure 159.

A sufficiently protracted excitation should lead to decrease in excitability and cessation of rhythmic activity, which is indeed usually observed upon the activity of the receptors and became known as "adaptation".

It might be thought that rhythmic activity of nerve centers occurs according to this plan. Any part of a neuron (the body of a nerve cell or the initial segment of a nerve dendrite) starts to increase its excitability to the level of the threshold of rhythmic activity. As soon as this threshold
275 is surpassed, impulses will pass centrifugally along the fiber. Thus, a slight increase or decrease in excitability in a very limited fiber segment will apparently stop the transmission of a rhythmic signal to the periphery.

Conclusions

In conclusion we would like to summarize the above with regard to spreading excitation*.

First of all, with respect to that group of phenomena generally called the "all or none" law, it should be pointed out that in the above theoretical considerations we dealt not with the rules of origin of excitation but with those regulating its spread. It has already been said that the very expressive term "all or none" originally meant to designate such a relationship between the magnitude of the stimulus and that of the response reaction, after which subthreshold stimuli caused no reaction ("none"), and above-threshold stimuli, independently of their magnitude, caused a maximum reaction ("all"). It has been shown that in relation to conducting fibers both statements are wrong. British physiologists have shown that in response to subthreshold stimulation a gradual response reaction is observed. It is true that Hodgkin (1938) states that there are no contradictions here with the "all or none" law, since this law is related only to a propagated effect but not to a local one. However, as has been shown, there is no basic difference between these two categories. A local effect spreads too, but this spreading is a decremental one. Thus, instead of the suggested "none" quite a considerable effect is observed.

The other half of the "law", stating that above-threshold stimulation caused a maximal response ("all"), is also erroneous. We have shown that the local response may be double the propagation peak, to the value of which the above-peak potential drops in the course of its movement. From this it is clear that the running peak is not maximal, and in reality we are dealing with a peculiar mechanism which is responsible for two adaptations, quite important for the activity of the nerve fiber.

The first is the threshold by which the nerve insulates itself from small stimuli by the fact that the subthreshold excitations arising in response to these stimuli may spread only with decrement and therefore fade away. Thanks to this adaptation, the nerve in the normal organism is practically insensitive to any natural stimuli along its entire length, except the propagating electric wave which is many times higher than the threshold.

The second adaptation is the automatically regulated dimension of the propagation peak. The value of this peak on the one hand is always considerably higher than the threshold, thus ensuring an undisturbed passage of the impulse through the entire fiber. We have seen that only under abnormal conditions of lowered excitability, the dimensions of the peak and of the threshold come close to each other and bring about conditions during
276 which a block may easily appear. On the other hand, the magnitude of the propagation peak is many times less than the possible maximum, thus protecting the nerve from residual damage which may arise as a result of the passing excitatory wave. This may probably explain the relative tirelessness of the nerve, described by Vvedenskii.

We have given in detail the theoretical considerations by which we propose to explain a number of known facts connected with the appearance

---

* A very good review of our studies on the theory of "gradual" conduction, published in Polish by Brutkowski (1956), has recently appeared in the foreign literature. In this review there are interesting comparisons between our theory and that of Hodgkin.

and conduction of nerve impulses, and which comprise the theory of "gradual" excitation. The latter is based on two postulates: 1) electric mechanism of transmission of the nerve impulse; 2) the statement that at each point of a fiber the magnitude of the local electric reaction is in a gradual dependence on the strength of stimulus and may be graphically expressed by an S-shaped curve. The theory of "gradual excitation", constructed only on these two principles, does not need any additional hypotheses on explosion-like response reactions of the living protoplasm. This theory leads to a number of conclusions, most of which are known as a result of purely empirical studies. The following belong to these conclusions:

1) Presence of a gradual subthreshold reaction;

2) Spreading of this reaction with decrement;

3) Presence of a threshold of spreading excitation;

4) Initial spreading of the above-threshold excitation with increment, until a certain constant level is reached;

5) Automatic regulation of the constant level of propagating excitation;

6) Appearance of rhythmic spontaneous activity of the fiber after increase in its excitability above a certain level;

7) Spread of excitation with decrement on lowering excitability below a certain level;

8) Obligatory alternation of absolute and relative refractory periods on passage of an excitation wave and during narcosis;

9) Increase in level of threshold potential, and simultaneous decrease in the level of the propagation peak during the relative refractivity and initial stages of narcosis.

In addition to explaining these already known facts the theory developed here made possible the forecasting and experimental confirmation of the following phenomena:

1) Appearance of a local electrical response reaction of greater magnitude than the propagation peak, after a sufficiently strong above-threshold excitation;

2) Spreading of this above-peak potential with decrement until dimensions of the constant-value propagation peak are reached;

3) Synchronization by electric excitation of disordered rhythm of active nerve fibers.

Agreement between all these theoretical conclusions and experimental data serve to confirm the validity of our theoretical considerations.

Part V

277 EXCITABILITY AND ITS MEASUREMENT

Chapter 1. Excitability Constants

Determination of Excitability Threshold

At the beginning of the book it was said that excitability, i.e., the ability to respond by one or other reaction to external stimuli, is one of the most characteristic properties of living matter. When the organism dies, excitability disappears altogether. That is why measurement of excitability has been used for many, many years in order to study the effect of different agents on a living organism. This still remains the most characteristic index of the tissues studied*.

Electric current is usually employed as the stimulus in quantitative evaluation of excitability. Either a mechanical effect (contraction of muscles), or the appearance of a moving wave of excitation (in the case of nerve and muscule fibers) is studied as the response reaction.

From the point of view of the theory of "gradual" excitation developed here (see Part IV), we do not see any basic difference between local and moving excitation, and all the rules of spread of an impulse have been deduced by us from the properties of the local gradual response, the dependence of which on the strength of excitation may be graphically illustrated by a smooth S-shaped curve. From our definition of excitability, it is obvious that it should be expressed by the ratio between strength of stimulus and the value of the local response. Such a difinition is the most general, since it relates to both conducting tissues (nerve, muscle) and nonconducting (glandular, epithelial, etc.). Therefore in the case of the nerve, for example, it seemed logical to measure excitability by the value of local electric reaction. However, great difficulties exist in the quantitative study of local effects on the nerve fiber. Therefore, such a direct measurement of excitability must be given up and instead the threshold of the moving impulse must be used as a criterion for evaluation of excitability. This can be easily and precisely determined.

278 One should have a clear picture of the relationship, from the point of view of the "gradual" theory, between the local electric reaction and the threshold of spreading excitation. To elucidate this question we shall turn to Figure 137 (see page 229). Here S-shaped curves are shown, characterizing fibers differing from each other only by the value of excitability. The higher the curve is located the stronger its response reaction to the same strength of stimulation, consequently the higher its excitability. As long as the excitability of the fiber is so high that its curve intersects with the

---

* As an index of the physiological state of a conductor, Vvedenskii suggested the so-called "lability"—a value characterizing the speed of excitation processes. While not denying the usefulness of this index, we do not use it to evaluate the state of a fiber, because the method of measuring lability has not yet been perfected.

bisectrix of the coordinate angle, the fiber is capable of nondecremental conduction (curves I-IV). As soon as all points of the curve fall below the bisectrix, nondecremental conduction ceases and the fibers are capable only of a local reaction (curves V-IX). Let us remember that the first intersections of the curves with the bisectrix determine the value of thresholds (oh = ah, oi = bi, oj = cj). The second intersections determine the value of the propagation peaks (gp, fn, em). The figure shows that with transition of the curve from position I to II to III, and to IV, the value of thresholds increases progressively with decrease in excitability of the fiber, acquiring the values of oh and later of oi, ok, and finally ol. Conversely, with increased excitability the threshold decreases. Thus, changes in threshold levels will always reflect the change in excitability of the fibers, but there is no strict inversely proportional relationship between these values. Consequently, on studying the change of thresholds, the direction of change in excitability may be judged. The relative value of shifts may also be described but there are certain reservations regarding accurate quantitative evaluation. This is one of the drawbacks to measuring excitability of the fiber by the threshold.

It has already been mentioned that with decreased excitability of the fiber, its curve, decreasing more and more, will fall below the bisectrix. When this happens, excitability as determined by the threshold of the conducted response will equal zero, since the fiber whose condition was characterized by curves V, VI, VII, VIII and IX will preserve its ability to respond only to a stimulation by local effect. This is the second basic disadvantage of this method. However, this is so far the only method of determination of excitability, and in the absence of others it must be used despite its shortcomings.

In the past century the only index of excitability was the threshold voltage of excitatory current. But from the beginning of the present century it became obvious that for the appearance of excitation under certain conditions, not only the voltage of the excitatory current, but also the duration of its action, is of importance.

Relationship between Threshold Voltage, or Intensity of Stimulation Current, and the Time of its Action

More than 150 years ago the famous physicist Volta (1803) demonstrated an inversely proportional relationship between the capacity of the condenser used for obtaining the threshold stimulation, and the voltage of the necessary electric current, in the case of sensitivity of the skin of the human finger to electric current. This important observation remained unnoticed until the end of the last century. The so-called law of excitation of DuBois-Reymond was predominant. According to this the value of the
279 threshold stimulus depends neither on voltage nor on the duration of current, but only on the changes in its intensity. Due to the authority of DuBois-Reymond, this law remained predominant for more than 50 years, although Fick (1863), using a nerve of the mollusk Anodonta and the calf muscle of the frog, and Engelmann (1870) using the ureter of the rabbit, have shown that not only the intensity of the current voltage, but also the time of its action play a certain role in determining the value of electric excitation

threshold. The turning point in development of the problem was without doubt the studies of Hoorweg (1892), which appeared much later but were very accurately executed. This investigator, working exclusively with human muscles, showed that with sufficiently small capacities of the condensors, the discharges of which were used as stimuli, the intensity of threshold electric stimuli (i) is inversely proportional to these capacities (C) and therefore also to the time of discharge, since the latter is proportional to capacity. On more prolonged action of the current, the threshold intensity no longer depended on the capacity of the condenser, or, which is the same, on the time of discharge. These relationships were expressed by Hoorweg, by the empirical formula

$$i = \frac{a}{C} + b, \qquad (1)$$

where i is the intensity of the current*, C is the capacity of the condenser and a and b are constants.

Later Weiss (1901a-1901d) experimented with excitation of nerves of the frog, toad, tortoise and man, by very short rectangular current pulses, and arrived at the same formula. This expressed the relationship between the intensity of the threshold current and the time of its action. According to his data

$$i = \frac{a}{t} + b, \qquad (2)$$

where i is the intensity of the current*, t the time of its action and a and b are constants.

The applicability of this empirical formula to different tissues was confirmed by a number of investigators. In addition, attempts were made to give it a theoretical basis, starting from the concept of the cell as an electric condenser (Chagovets, 1903; Ebbecke, 1927; Hill, 1935, and others).

At the same time, Nernst (1899, 1908), starting from the hypothetical concept of semipermeable membranes on the surface of fibers, and also assuming that excitation should appear when the concentration of ions on these membranes reached a certain definite value under the influence of electric currents, stated a formula according to which

$$i = \frac{a}{\sqrt{t}} \qquad (3)$$

This formula differs basically from those of (1) Hoorweg and (2) Weiss in that here the intensity is inversely proportional to the square root of time, while there it is simply inversely proportional to time. Nevertheless,
280 Nernst produced copious experimental data (of his own and from other authors), designed to convince the reader that he and not Hoorweg and Weiss was right.

---

* In reality it is not the intensity of the current which is measured, but the voltage, which is proportional to the intensity of current at a constant resistance. Therefore in certain cases we shall, in the formula of Hoorweg, use voltage instead of i. In that case the formula will be as follows: $v = \frac{a}{C} + b$.

Odd as it may seem, both formulas entered into text books where they co-exist, notwithstanding the obvious contradiction between them (see for example, Rubinshtein, 1947). The formula of Nernst, on the basis of the membrane theory, is apparently adequately confirmed by the experiment. At the same time it is indicated that in practice, especially for very short time intervals, the formula of Hoorweg is the more accurate. The reader may remain confused: what is the truth of the matter? Only after an accurate empirical solution of this problem can a correct theory be constructed.

One of the tasks we took upon ourselves (Nasonov and Rozental', 1953) was an attempt to determine empirically the relationship between intensity of electric stimulus and the time of its action. Another task, closely linked with the first, was the attempt to elaborate a rational method to measure excitability of tissue because, as we shall try to show later, the widely used chronaximetric method suffers from a number of basic shortcomings.

Physiological Significance of Constants a and b in the Formula of Hoorweg-Weiss

Let us start from analysis of the formula of Hoorweg-Weiss ($i = \frac{a}{t} + b$). At sufficiently small time intervals the ratio $\frac{a}{t}$ becomes so large in comparison with b, that the term b may be ignored. Then the formula will be transformed into a simple inverse proportion:

$$i = \frac{a}{t} \tag{4}$$

On the other hand, if the time intervals are sufficiently large then $\frac{a}{t}$ becomes so small in comparison with b that this ratio may be neglected, Then i becomes a constant value:

$$i = b. \tag{5}$$

Thus, the general formula of Hoorweg (1) expresses two laws. One is applicable to the region of specific short time intervals, while the other—to the region of more prolonged ones.

From equation (5) follows the physiological meaning of constant b. This constant numerically equals the threshold intensity of the current (or threshold voltage) in the region of prolonged time intervals. It characterizes excitability which does not depend on time. We shall from now on denote it as the protracted threshold of excitability.

Further, from the formula $i = \frac{a}{t}$ it follows that $a = it$, or that a is a product of the threshold intensity of current at the time of its action. If the independent variable $t = 1$, then $i = a$. From this equation follows the physiological meaning of constant 'a'. This constant numerically equals the threshold intensity (or voltage) of the current which will cause excitation for a certain given time of action accepted as a unit, in the region of suf-
281 ficiently short intervals. Consequently, constant 'a' characterizes

excitability depending on time. We shall further call it the "short-term" threshold of excitability.

These two constants characterize excitability of the conducting fiber if the latter behaves according to the formula of Hoorweg. It is not difficult to find these constants. We shall show later that under the influence of these or other agents, both characteristics of excitability may change to a sufficient degree, independently of each other and not proportionally to each other, and that in order to understand the effect of various factors on excitability of the conducting tissues it is necessary to study simultaneously the dynamics of changes in both a and b. So far this has not been done. Since the first studies by Lapicque and his co-workers, only the protracted threshold of excitability, called by Lapicque the rheobase, was studied, as well as the quantity introduced by him—chronaxy, the value of which causes a number of serious doubts (see below).

The short-term threshold of excitability may be expressed in volts per unit of time. But it may also be expressed in fractions of a second per unit of voltage. Indeed, in the formula $i = \frac{a}{t} i$ may be made to equal unity and then a = t (numerically). In this case the constant a may be defined as the threshold time for the duration of which a current intensity (or voltage) chosen as a unity causes tissue excitation within the range of short-time intervals. This is another way of expressing the constant 'a' as in physics. Action speed, for example, may be expressed either by the length of the path travelled in a time unit or the time necessary for traversing a unit of path. Correspondingly, we shall further call the constant a either the short-term threshold of excitability (starting from 'a' as proportional to the threshold voltage of current at constant time), or threshold time of excitation—T (starting from 'a' as proportional to the threshold time at a constant voltage).

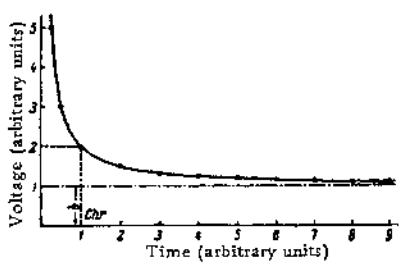


FIGURE 161. Voltage—time curve plotted according to the formula $i = \frac{a}{t^n} + b$, where a, b and n are equal to 1. Chr--the chronaxy.

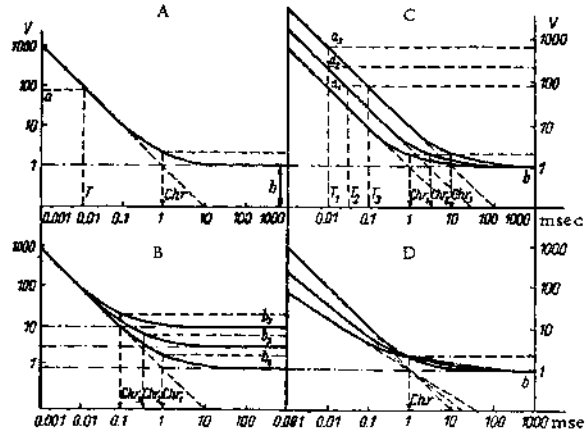


FIGURE 162. Logarithmic voltage-time curves, corresponding to the empirical formula $i = \frac{a}{t^n} + b$ (according to Nasonov and Rozental', 1953)

A—curve constructed according to the formula where a, b and n equal 1; B—change of the curve on varying constant b; C—the same, varying constant a; D—the same, varying constant n. Chr—chronaxy; a—threshold of short-term excitability; b—threshold of protracted excitability (rheobase); T—threshold time of excitation.

Usually the relationship between intensity and time of excitation is graphically expressed in the form of a curve, the abscissa of which shows
282 the time of action of electric current, and the ordinate its intensity. Such a curve is an equilateral hyperbola, the left wing of which approaches asymptotically the ordinate while the right wing strives to a straight line parallel to the abscissa, removed from it by the distance b (Figure 161). The inconvenience of such a presentation is that on the ordinary scale it is only possible to depict a small section of the curve. It is therefore much more convenient to plot on the coordinate axes, not the time and intensity (or voltage), but their logarithms. Then the intensity time curve (or voltage-time curve), corresponding to the formula of Hoorweg-Weiss acquires the form given in Figure 162A.

It will be seen later that the right wing of such a logarithmic curve strives to a horizontal straight line located at the level of log b, and the left wing strives to a straight line sloping at 45° to the abscissa. On such a graph (Figure 162A) the abscissa encompasses an interval of excitation from 0.001 to 1,000 milliseconds, i.e., differing from each other millionfold. The ordinate shows the logarithms of voltage from 0.1 to 1,000 volts, i.e., values differing from each other 10,000 times; while the ordinary graph (Figure 161) shows only several hundred units discernible to the eye.
283 Another great advantage of logarithmic curves is that the shape of the curves does not change according to the scale of units plotted on the coordinate axes; only the position of the curve changes.

The logarithmic curves clearly illustrate the value of the constants a and b in the formula of Hoorweg (Figure 162A). Figure 162B shows curves in which only the constant b varies. As a result, the right hand rectilinear wing of the curve shifts parallel to itself. This means that in the region of sufficiently short time intervals everything remains as before, while in the region of prolonged intervals the excitability of the fiber varies.

In Figure 162B only 'a' varies. In this case, the left hand rectilinear wing of the curve (at a slope to the abscissa) also shifts parallel to itself, while the position of the right wing does not change. This means that excitability of the fiber changes on excitation with short currents, while on excitation with more prolonged currents the excitability remains constant. Such cases are met with in practice.

Gradient Factor (Constant n)

The applicability of the formula $i = \frac{a}{t} + b$ to these or other specific cases is very easy to check if the curves are drawn to a logarithmic scale.

In the region of short-time intervals $i = \frac{a}{t}$. The logarithms of this equation gives:

$$\log i = \log a - \log t \qquad (6)$$

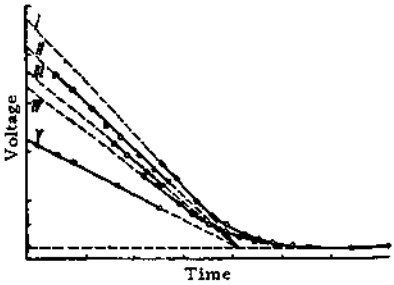


FIGURE 163. Logarithmic curves of voltage-time of 5 different tissues (I-V) (according to Nasonov and Rozental', 1953)

I—nerve of the biceps muscle of man, n = 1.12; II—frog nerve, n = 0.92; III—muscle of leech, n = 0.85; IV—skin sensitivity of man, n = 0.78; V—frog stomach, n = 0.53. For explanations see text.

If the logarithms of time (log t) are plotted on the abscissa, and the logarithms of intensity (log i) on the ordinate, equation (6) will be expressed by a straight line oblique with respect to the abscissa.

Since the coefficient before log t equals unity, the slope of this straight line should be 45°; consequently such straight lines should cut off segments of equal length on the coordinate axes (tan 45° equals 1).

At the same time, in the region of sufficiently long time-intervals of excitation i = b. Using the logarithms of this equation we obtain log i = log b, which is also expressed by a straight line parallel to the abscissa at level of log b. It is quite obvious that these two straight lines corresponding to sufficiently short and sufficiently long durations should be joined by a plane segment. The logarithmic curve thus obtained is shown in Figure 162A. In the logarithmic form all the known curves of strength-time asymptotically approach two straight

lines without exception: to the right—parallel to the abscissa, and to the left—inclined at a certain angle to it (Figure 162A). However, the slope of this left-sided straight line does not always satisfy the formula of Hoorweg-Weiss in which this slope, expressed by the tangent of the angle (ratio of segments on the ordinate and on the abscissa), should equal 1.

Figure 163 shows a group of different curves of voltage-time obtained by us reduced to a common rheobase and drawn close to each other, so that the beginnings of their inflection coincide, for the sake of clarity. This treatment does not change the angles of slope of their left hand straight
284 segments. Each of the given curves (I-V) has its own slope*.

What does this slope mean? How should this reflect on the empiric formula corresponding to these curves?

As already stated, the left hand part of the logarithmic curve of intensity-time according to Hoorweg-Weiss, should be expressed approximately by the equation (6). We have also stated that this is a straight line equation at a 45° angle to the abscissa, since the slope of the straight line is determined by the coefficient of proportion of the independent variable (t). In the given case this coefficient represents the tangent of the slope of the straight line, which here equals one. We have thus been convinced that this slope differs in various cases and the tangent of its angle may vary within the limits of 1.0 to 0.5. Consequently this part of the logarithmic curve should correspond to the equation

$$\log i = \log a - n \log t$$

and in the nonlogarithmic form

$$i = \frac{a}{t^n} \tag{7}$$

Then in the complete form the intensity-time curve should be expressed by the following equation:

$$i = \frac{a}{t^n} + b \tag{8}$$

Thus, we reach a very interesting conclusion, solving the contradiction between the formula of Hoorweg-Weiss on the one hand, and that of Nernst on the other. Both formulas are only particular cases of a more general rule expressed by equation (8).

In this equation the constant n may acquire different values. For a number of tissues, for example nerves and skeletal muscles of vertebrates, it is close to one, and we approach very close to the formula of Hoorweg-Weiss. In other cases—nerves and muscles of certain invertebrates, smooth muscles of vertebrates, skin receptors of man, etc., this value may approach 0.5. In that case a relationship close to Nernst's formula takes place.

285 It is quite obvious that all the curves given in Figure 163 cannot coincide at any chosen scale, due to the different slope of the left wing. However, Lapicque (1926) in his book states that with a proper choice of the

---

* On determination of the slope of the curves by a logarithmic graph, errors within the limits of ± 10% are possible.

time scale, all the strength-time curves can be superimposed on each other at all their points. In order to prove this contention he chose from among his extensive data 6 intensity-time curves in which the slopes of the rectilinear left wing ("n") in the logarithmic scale were approximately 0.5. However, in other tissues studied by him the values of n deviated from 0.5. Figure 164 shows two logarithmic curves of voltage-time plotted according to the data of Lapicque and reduced to the same rheobase. It is obvious that superimposition of these curves does not occur here.

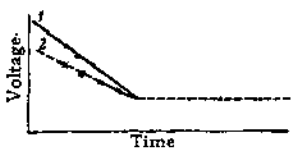


FIGURE 164. Logarithmic voltage-time curves for the vine snail (Helix pomatia) (according to Lapicque, 1926)

1--heart muscle; n = 0.75;
2--locomotor nerve, n = 0.5.

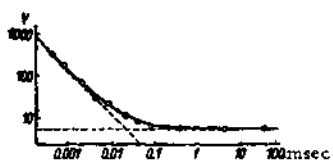


FIGURE 165. Ideal logarithmic voltage-time curve corresponding to the formula $i = \frac{a}{t^n} + b$ (continuous line) (according to Nasonov and Rozental', 1953)

The points are values actually obtained for human biceps; n = 1.

The physiological significance of the constant n can be most clearly demonstrated by a specific example. Let us assume that in the region of very short intervals of current action we reduce its duration 4-fold. If we accept that each time, in order to obtain excitation waves, a 4-fold stronger current is necessary, this will mean that a simple inverse proportion occurs ($i = \frac{a}{t}$) and that n = 1, as assumed by Hoorweg, Weiss, Hill and others. However, it may be that on each 4-fold reduction of the time of action current it is necessary to increase the intensity not 4-fold, but 2-fold. Mathematically this may be expressed by the formula $i = \frac{a}{\sqrt{t}} = \frac{a}{t^{0.5}}$, i.e., a relationship postulated by Nernst: (as has been shown, n may also have other values from 1.0 to 0.5). Consequently in the formula n expresses the slope of increase of threshold intensity of the current on reduction of its time of action. Graphically, on the logarithmic curve of intensity-time, the value n determines the slope (the tangent of the angle) of the asymptote to which the curve approaches. In Figure 162B only n changes in the formula $i = \frac{a}{t^n} + b$. Correspondingly, only the slope of the asymptote changes. The constant n will be called the curve incline factor. Its practical determination presents no difficulties. Obviously, the common error of Nernst and Lapicque, and of Ebbecke and Hill was the conviction that the same relationship exists for all tissues as between the intensity of current and the time of its action. This was required by the theory from which they started. However, as has been shown above, this relationship differs (the constant n varies from 0.5 to 1). It is interesting that the necessity to introduce an exponent of the nth power varying from 1 to 0.5 into the empirical formula of Hoorweg for time, was already indicated by the American investigator Cole in 1933, and independently by the French investigator Colle, (1933).

However, the results of both authors remained unnoticed, probably because they complicated the problem to a great extent and refuted both the concentrational (Nernst), and the condenser theories of excitation (Ebbecke, Chagovets, Hill). These studies, for example, were not included in the review of chronaximetry written in Russian by Uflyand (1938). They were also unknown to us at the time when we published our papers in which we expressed for the first time our views on the curve incline factor n (Nasonov and Rozental', 1953). We assume that if in 3 papers the authors reach the same conclusions at different times and quite independently from each other, this indicates that these conclusions are correct.

Table 50

Threshold voltages of intensity-time curves of human biceps (according to Rozental' and Filippova, 1957)

Capacity of condenser (in μf)	Subject No. 1			Difference (in %) according to formula $\frac{v_2-v_1}{v_1}100$ for subjects:				
	v_1, from Hoorweg's formula	v_2, experimental	Difference (in %) according to formula $\frac{v_2-v_1}{v_1}100$					
	voltage			No. 2	No. 3	No. 4	No. 5	No. 6
90	—	6.6	—	—	—	—	—	—
30	—	6.6	—	—	—	—	—	—
5	7.2	7.2	+0.4	— 0.3	+ 6.6	+4.4	+ 4.3	— 3.9
2	8.0	8.2	+2.5	— 1.6	+ 2.3	+2.0	+ 2.6	+ 1.5
1	9.5	9.4	—0.1	+ 1.8	+ 2.2	+3.6	+ 6.1	+ 2.6
0.5	12.4	11.2	—0.9	—13.3	+ 7.8	+0.6	+ 7.5	— 9.7
0.2	21.0	20.0	—5.0	— 6.3	+ 8.1	0.0	— 8.0	— 3.1
0.1	35.3	34.0	—3.7	— 3.6	— 0.3	—9.8	— 2.0	— 2.7
0.05	64.0	62.0	—3.1	— 7.3	— 2.2	—2.6	— 2.2	— 8.4
0.02	150.0	150.0	0.0	+ 7.2	+ 2.2	0.0	+ 2.9	— 9.2
0.01	293.6	310.0	+5.6	— 8.2	— 0.9	—8.6	0.0	+ 9.4
0.005	—	—	—	—	+12.1	—	—12.9	—16.8

To what extent does the suggested empirical formula, $i = \frac{a}{t^n} + b$ coincide with the actual curves of strength-time?

It has already been stated that in the regions of very short, as well as in those with prolonged time intervals, any logarithmic curve of intensity-time is transformed into 2 straight lines: one inclined to the abscissa and one parallel to it. Here there is close agreement with the formula. The curvilinear part connecting the two straight lines also corresponds exactly to the formula in certain tissues. Thus, Figure 165 shows an ideal curve corresponding to the formula $i = \frac{a}{t^n} + b$, and points are shown obtained with
287 the human biceps. Results agree within the limits of experimental error.

Table 50 shows values of threshold voltages for various times excitation current for the biceps of 6 (human) subjects studied (Rozental' and Filippova, 1956). These values were compared with those calculated according to Hoorweg's formula. This could be done because in all the muscles studied, the coefficient n was close to 1. For tissue no. 1, the absolute theoretical and experimental values are given, as well as the percentage difference between them. For the muscles of the remaining 5 experimental tissues, only the percentage deviations are given. The experimental and calculated figures agree closely. In the majority of cases the difference is less than 10%.

For the frog sciatic nerve the correlation between experimental and theoretical figures is not quite so close, as a rule. In the transitory part of the curve, deviations are observed which are probably caused by the fact that the nerve consists of fibers with different properties, in which the intensity-time curves intersect with each other. Anomalies of this kind were described in detail and studied by Lucas (1907), Lapicque (1926), Rushton (1931, 1932, 1935) and others.

Chapter 2. Measurement of Excitability.

Method of Measuring

From the previous chapter it is clear that to measure the excitability of the conducting fiber completely, it is necessary to determine 3 constants—a, b and n. However, no one has done this until now. Instead, for the characterization of excitability, the method of chronaximetry introduced by Lapicque was used, consisting of determination of the constant b (rheobases) and of the value suggested by Lapicque, i.e., chronaxy, supposedly serving as the time factor.

Determination of the three basic constants of excitability presents no difficulties. In practice only slight changes in normal chronaximetry are required. It is necessary to increase the region of the short intervals of excitation somewhat and to increase the store of electric voltage correspondingly.

For these purposes an instrument was used, the scheme of which is illustrated in Figure 166. The time of condenser discharge is determined by the formula $t = cR$, where c is the capacity of the condenser and R is the resistance of the chain. We did not consider it possible to shorten the time intervals by reducing the capacity below 0.001 microfarads, since by doing this the accuracy of measurement sharply decreased. We reduced resistance (R), substituting the Lapicque shunt by a 100 ohm shunt. This shortened the time of discharge of the condenser 100-fold, thanks to which the recalculation for time was achieved by multiplying the number of microfarads, not by 4, as required in the case of the Lapicque shunt, but by 0.04. The resistance of the tissues (thousands and tens thousands of ohms) always exceeded by so much the value of 100 ohms that some fluctuation in the
288 dimensions of this resistance did not appreciably affect the speed of discharge of the condensers. The choice of capacities was from 600 to 0.001 microfarads, which made it possible for us to vary the time of discharge from 2.10^{-2} to 4.10^{-8} seconds. There was obviously no great distortion

with the shortest discharges, since the rectilinear distribution of the points on the logarithmic curve was observed up to the very last measurements. The maximal voltage used was about 400. A battery of dry elements or a kenotronic rectifier served as source of current.

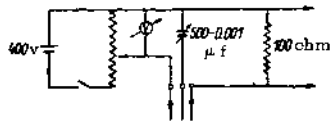


FIGURE 166. Scheme of apparatus for the determination of excitability constants (according to Nasonov and Rozental', 1953). For explanation see text.

In addition to the 100 ohm shunt, a resistance of 50 k ohm was introduced into the chain in series. It is also recommended that the distance between the electrodes be about 1.5-2.0 cm. The last two measures were necessary to minimize the changes of threshold intensity of the current, after changes of resistance of the nerve segment under investigation. If, however, the measurements were performed in man with the use of skin electrodes, the resistance to the current brought to the nerve was so much increased by the skin and other tissues that the serial resistance of 50 k ohm was no more necessary. The theoretical basis for these two measures will be given in Part VI of this book.

Using a number of tissues (nerve-muscle preparation, myocardium and stomach of frog, leech muscles and others) parallel curves were determined with the Lapicque shunt and with our shunt. The results showed that the shape of the curve does not change on transition from one shunt to the other (Figure 167). The excitability curves obtained in man on excitation through the skin were an exception.

Measurement of the excitability constants was performed in the following way. First of all the whole voltage-time curve was determined and stated in logarithmic form (Figure 168, 1). For determination of the constant n, the left sloping asymptote was continued to the intersection with the coordinate axes. The ratio between the value of the segment cut off on the ordinate to that on the abscissa gave the value of n. In the case described this ratio was close to 1 (n = 0.97).

289

We found that for the majority of tissues (nerves and skeletal muscles of vertebrates) the value of n is close to 1 (Hoorweg's formula: $i = \frac{a}{t} + b$ is valid here), whereupon during the action of various agents, the slope of the left wing of the logarithmic curve does not change (see Figures 168, 170 and 171). Consequently, in the case of these tissues it is not necessary to determine the constant n.

Constant b (Lapicque's "rheobase") was determined in the usual way. Attention will be drawn to determination of the threshold of short-term excitability or constants a, since it raised the greatest number of problems in the discussion that took place regarding chronaximetry (Uflyand, 1954; Abrikosov and Darkshevich, 1954; Kiselev, 1954; Navakatikyan, 1954; Nasonov and Rozental', 1955).

As already stated, in the region of sufficiently small time intervals, it may be assumed that $i = \frac{a}{t}$ or $a = i \cdot t$ (or $a = vt$).

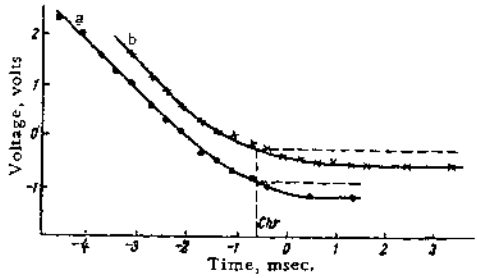


FIGURE 167. Logarithmic curves of voltage-time in nerve-muscle preparation of a frog.

a--measurements performed with a 100 ohm shunt; b—measurements performed with the Lapicque shunt; Chr--chronaxy.

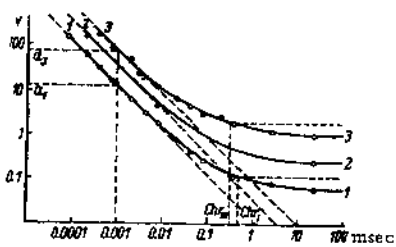


FIGURE 168. The effect of 5% ethyl alcohol on a nerve-muscle preparation of frog (according to Nasonov and Rozental', 1953)

Logarithmic curve of voltage-time: 1—before narcosis; 2--the same preparation 38 minutes after onset of narcosis; 3—same preparation 1 hour 36 minutes after onset of narcosis; n = 0.97. Remaining legend as in Figure 162.

Transforming Hoorweg's formula $v = \frac{a}{t} + b$, we obtain $a = (v - b)\,t$. If v exceeds b 100-fold, then discarding b in this formula creates an error in the calculation not exceeding 1%, which is insignificant, being considerably lower than the experimental error. Then constant a will be determined by the product of threshold time and threshold intensity (or voltage). We suggested using the product of the threshold number of millivolts and the threshold number of milliseconds as the general unit for measurement of a (Nasonov and Rozental', 1955). We shall show the determination of a in a specific example of the intensity-time curve of a frog nerve under normal conditions, and after the action of alcohol (Figure 168). It follows from Table 51 that the rheobase of this nerve is 0.059 volt. A 100-times greater value will equal 5.9 volts. Consequently, if a is determined at more than 6 volts the error will be less than 1%. On this basis we chose a time-interval of excitation which was so short that it requires a threshold voltage not less than 6 volt. Taking 0.001 millisecond, for example, we find that a threshold voltage of 12 volts is necessary. Since a = vt the value of a equals 12,000 millivolts x 0.001 milliseconds = 12 millivolt milliseconds. That is all that is required for determination of the constant a. As in the determination of the rheobase, this takes no more than a minute. In view of the fact that a = vt we shall

obtain the same figure, 12 millivolt millisecond, determining a at any higher voltage.

Table 51

Effect of 5% ethyl alcohol vapor on excitability constants of frog nerve and on its chronaxy (according to Nasonov and Rozental', 1953)

Constant	Before narcosis (see Figure 168, curve 1)	After 1 hr, 36 min of narcosis (see Figure 168, curve 3)	Changes in initial value (in %)
Increment (n)	1.0	1.0	0
Protracted threshold of excitability (in volts)	0.059	0.98	+1560
Short-term excitability threshold (in mvolt msec)	12	73	+ 500
Chronaxy (in msec)	0.5	0.32	— 36

290 Subsequently the nerve was subjected to the action of 5% alcohol vapors. Its excitability decreased and correspondingly, the curve successively assumed positions 2 (after 38 minutes of the narcotic action) and 3 (after 1 hour, 36 minutes). As seen from Table 51 and Figure 168 the rheobase became equal to 0.98 volts, which meant that the protracted threshold of excitability increased 16.6-fold. For determination of the short-term threshold of a, the same time was applied (0.001 millisecond) and its threshold voltage found. Now the latter will be not 12 volts but 73 volts. Consequently, the short-term threshold of excitability will now be 73,000 millivolts x 0.001 milliseconds = 73 millivolt milliseconds. The constant a increases 6-fold (less than constant b!).

From the above example it will be seen that determination of the constant a is very simple. It becomes somewhat more complicated when dealing with a curve corresponding to the formula $i = \frac{a}{t^n} + b$. In that case, for short-time curves $a = it^n$, and therefore to obtain the numerical value of a in the same units, the number of millivolts should be multiplied by the number of milliseconds to the nth power.

In the majority of cases the purpose of the study is not to compare excitability of different tissues, but to study changes of excitability in the same tissue following the action of different agents.

The simplest way of determining a is not in absolute units (millivolt, millisecond) but in relative ones, assuming the initial value of a to be 100%*. For this purpose, in the region of sufficiently small time-intervals, of excitation (where v is larger than b, for example, 100-times) any convenient time period may be taken (for example, 0.001 millisecond). This was accepted as a unit and the threshold number of volts determined. In our case it was 12 volts. This initial number we assumed to be 100% and we subsequently studied the dynamics of this value either in the course of

---

* Determination of a in relative units of measurement was suggested by us in our 1953 studies (Nasonov and Rozental').

experiment, or during various pathological processes, finding the threshold number of volts by using one and the same time period (0.001 millisecond).

The rheobase may also be expressed in per cent. In our example (see Table 51), after the action of 5% alcohol vapor for $1\frac{1}{2}$ hours, the protracted threshold of excitability increased by 1,560%, while the short-term
291 excitability threshold increased only by 500%. These values often changed not directly in relation to each other but sometimes even in inverse proportions.

Many contemporary physiologists firmly believe that it might be possible to separate the constant determining excitability of nerve or muscle fibers and the time constant (or factor) determining the time necessary for appearance of excitation at a certain current intensity. This viewpoint is basically wrong. In reality there is not an excitability factor and a time factor but two excitabilities: one, independent of time and characteristic for long intervals of current action, and the second, connected with the first by gradual transitions, depending on time and characterized by short intervals of current action. This short-term excitability is characterized by constant a. It is proportional to the threshold-time of the current action at a constant intensity; consequently it is also a constant time. It is also proportional to the threshold intensity of the current at constant time, consequently it is also an excitability constant for short-time intervals.

The method of determination of a just described is the most accurate, because this value, like the rheobase, is determined directly by one measurement only. In certain cases, however, the value of the rheobase is so high that the instrument will not allow the determination of a by a voltage exceeding 100-fold that of the rheobase. In that case, b cannot be disregarded in equation $a = (i - b)\,t$, without introducing a considerable error, and it is therefore necessary to subtract the value of the rheobase b from the threshold voltage v (i). In this case the error of determination of a increased considerably. Consequently, voltages higher than the rheobase should be used only in cases of extreme necessity.

Method of Determination of Excitability of Human Muscles

In studying excitability of human muscles (Rozental' and Filippova, 1957) the above-described apparatus was used (Figure 166), but without the serial 50 k ohm resistance. This resistance was actually replaced by the high resistance of tissues through which the current passed on its way to the nerve. The excitability thresholds of nerves and muscles on the intact organism are much higher than those of the isolated tissues, but thanks to the 100 ohm shunt a complete intensity-time curve may be obtained, using a voltage of the current source of 400-500 volt, in the intact organism as well. In the majority of condenser chronaximeters now in wide use for determination of the rheobase, a pulse of constant current is used. Such a determination is usually accompanied by pain sensations (Uflyand, 1938). In experiments by Rozental' and Filippova the determination of the rheobase by use of condenser discharges of long duration was absolutely painless.

The method of so-called unipolar excitation in which the acting electrode is a small differential one, applied to the skin at the motor point

(motor point—a narrow region of maximal excitability). This is related to the peculiarities of distribution of electric current in the human organism.
292 The differential electrode is usually a round plate of chlorinated silver, 1 sq cm in area, wrapped in cotton and gauze and soaked with a physiological solution. In accepted chronaximetric practice the differential electrode is applied to the motor point in each measurement of excitability. This method has basic shortcomings. If the electrode is moved away from the motor point only by a few millimeters or pressed a little stronger, quite different values of excitability are obtained. The moisture of the electrode is also of importance. Stable results are obtained only by investigators with great technical experience. All these factors introduce a subjective element into the measurements which is very difficult to evaluate.

For the study of dynamics of excitability during short time periods (not more than 1 day) this method was modified. A chlorinated silver cup 1 square cm in area and 4 mm in depth was used as differential electrode. The cup was filled with cotton soaked in hot 2% agar-containing Ringer's solution. After the agar solidified, the motor point was found and the cup-electrode was fixed to it with adhesive tape. In the intervals between experiments the electrode was immersed in Ringer's solution. This modification of the electrode always ensured the same degree of moisture, the same degree of pressure, and tight fixation of the electrode to the motor point. This completely eliminated subjective errata in studying excitability. However, there is one less important shortcoming not eliminated by this method, i.e., the possible shift of the motor point. It is self-evident that in more prolonged studies, exceedings 1-2 days, this method is not applicable.

Comparison of Excitability of Various Tissues

The absolute values of excitability thresholds measured (a and b), depend to a large extent on the histological structure of the investigated tissue, since this determines the fraction of the electric current which penetrates the fiber and serves as the actual stimulus. Therefore, comparison of excitabilities of various objects is only of relative value, sometimes being quite impracticable.

Nevertheless, performing measurements under strictly standardized conditions allows comparison, with a certain accuracy, of excitability of various tissues.

Figure 169 shows voltage-time curves of 5 various tissues, and Table 52 shows the numerical values of their excitability constants. The tissues are arranged in order of increasing rheobase (threshold of protracted excitability), i.e., in order of decreasing excitability according to this constant.

In all the tissues, constant a was determined by the threshold time necessary to obtain a propagating excitation from 100 volts. In order to obtain the numerical value of a, the voltage in millivolts (100,000 millivolts) was multiplied by the threshold time in milliseconds*. This was done in all

---

* For the determination of a another voltage could also be used, for example, 150, 200 volt, etc. In all cases the same numerical value for a would be obtained, since a = it.

293 cases with the exception of the frog stomach, because here the steepness of constant n is close to 1 and a is calculated from the formula a = it. In the frog stomach n = 0.5, therefore the numerical value of a was determined from the equation $a = i\sqrt{t}$*. From Table 52 it will be seen that according to the short-term threshold of excitability a, all the tissues are arranged in this case as according to b.

Table 52

Excitability constants of various tissues (according to Nasonov and Rozental', 1953)

No. of curve (see Figure 169)	Experimental object	b (in volts)	Threshold time at 100 volt (in msec)	a (in mvolt·msec)	n	Chr (in msec)
1	Frog, sciatic nerve	0.036	0.00004	4	0.94	0.38
2	Frog, sartorius muscle	0.16	0.00017	17	0.92	0.90
3	Frog, heart muscle	0.19	0.0053	530	0.96	7.00
4	Leech, muscles	1.52	0.0060	600	0.89	1.05
5	Frog, stomach	—	0.14	37,500	0.52	—

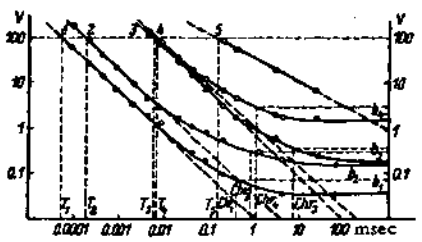


FIGURE 169. Logarithmic voltage-time curves of various tissues (according to Nasonov and Rozental', 294 1953)

1—frog nerve; 2—sartorius frog muscle; 3—heart muscle of frog; 4—muscle of leech; 5—frog stomach. Remaining legend as in Figure 162.

The fact that a is a measure of the threshold time of excitation follows from the formula a = it, since at a constant time t, a is proportional to i, while at a constant i, a is proportional to t. Figure 169 clearly illustrates this point. Here, a voltage of 100 volt was used as the constant v (i). A current of such a voltage should cause excitation in the frog nerve in 0.00004 milliseconds, in the frog muscle—0.00017 milliseconds, in the heart muscle—0.0053 milliseconds and in the muscle of the leech—0.0060 millisecond. Consequently, frog nerve is the most "rapid" of these tissues, and the muscles of the leech—the "slowest", according to Lapicque's terminology.

All that has been said above refers to tissues with a similar constant of n. Excitability in short intervals in such tissues may be compared by a. For tissues where n differs, it is impossible to compare short-term excitability only by a. Their voltage-time curves may intersect and the ratios of their velocities of reaction will differ, depending on the time intervals during which they are measured.

---

* The rheobase in the case of frog stomach was not determined since it is located in the region of durations of excitation longer than those for which the measuring instrument was devised.

Table 52 shows that the arrangement of the tissues according to the value of constant a does not always coincide with the series according to chronaxy, which, in the opinion of most contemporary physiologists, should serve as a time constant. It is quite obvious that chronaxy is not always a reliable index. We shall try to show later that chronaxy is not a time constant, as is usually thought, and we shall try to show the sources of error in chronaximetry.

Furthermore, attention is drawn to the fact that the tissues, quite arbitrarily chosen by us, differ much more in respect to constant a than b. Thus, in extreme examples the rheobase of frog nerve is 42 times smaller than that of leech muscle, while the short-term thresholds of excitability differ 150-fold. There is reason to believe that such relationships exist in the comparison of most tissues.

Finally, another result from the comparison of voltage-time curves of various tissues follows. Figure 169 shows that the left wing of the logarithmic curve of smooth gastric muscle (5) is inclined to the abscissa at a more acute angle ($n = 0.52$) than in other curves (in the case of heart muscle $n = 0.96$, for skeletal muscle, $n = 0.92$; Table 52). It follows from this that on further shortening of the stimulus time, the voltage-time curve of smooth muscles will intersect with the other curves. This obviously means that in the region of short intervals of excitation, stomach muscles are more sensitive and more "rapid" than heart and skeletal muscles. On testing their sensitivity by current of the same voltage, the threshold time for smooth muscles will be less than for striated muscles. The intersection with the curve for heart muscle will take place approximately at a duration of stimulus of 0.0001 milliseconds and a voltage of approximately 3,000 volt; that with the curve of skeletal muscle—at a duration of 0.00000001 millisecond and a voltage of approximately 1,000,000 volts. An appropriate instrument may be constructed to check these conclusions. This is comparatively easy to do with heart muscles.

At first sight this conclusion may seem paradoxical. But actually, it is logical. Subsequently, data will be given to indicate that the excitation in conducting fibers is determined not by the intensity of the current or by voltage, but by its energy. On this basis it can be shown that excitability of nerve elements, and of striated muscle fibers of vertebrates, depends to a large extent on duration of the stimulus by which excitability is determined. A sharply expressed maximum of excitability exists in the region of the optimal physiological time intervals (for frog muscles about 0.1 milliseconds). To the right and to the left of this maximum the excitability of the fibers drops very steeply (see Figure 204).

295 Smooth muscles do not possess this property, and in the region of sufficiently short intervals of stimulation their excitability does not depend on the time of action of the current. It follows from this that excitability and speed of reaction of skeletal muscles should exceed those of smooth ones only at the optimum time of action of the stimulating current. At very short, nonphysiological time intervals, these relationships are distorted.

Chapter 3. Chronaxy

Does Chronaxy Determine the Rapidity of Excitation?

Due to the studies of Hoorweg (1892) and Weiss (1901a-1901d), it became obvious that for excitation not only the current intensity, but also its time of action was of great importance. It became necessary to introduce a time constant into determination of excitability.

To estimate intensity-time curves Lucas (1907) used the threshold duration necessary to cause excitation at twice the threshold intensity. Lucas called this time the "excitation time". However, he later rejected the use of this value as a time factor.

Lapicque (1909) who borrowed this term from Lucas, gave it the name of chronaxy, which, when translated from the Greek, means "time value". According to Lapicque this value, together with the "rheobase" (a term also introduced by him) fully characterizes the excitability of a tissue, both as to intensity of excitatory currents and regarding the duration of its action. Since then chronaxy has become firmly established in physiological usage as a time constant, being considered as a measure of reaction-velocity of the tissue. It is sometimes said that chronaxy and rheobase are a measure of excitability.

In the literature critical remarks were often made regarding chronaximetry. However, it seems to us that they dealt with matters of secondary importance. They did not dispute the importance of chronaximetry as a method to measure the rapidity of appearance of excitation in conducting tissues (Cremer, 1929; Blair and Erlanger, 1933; Lazarev, 1934, 1939, 1947; 1947; Rubin, 1939; Anokhin, Maiorchik and Slavutsskii, 1945; Rubin and Fedorova, 1951).

The problem of chronaxy will first be considered from the mathematical point of view.

Chronaxy is defined as the time during which a current equal in intensity to twice the rheobase should act on a tissue in order to cause threshold excitation. We shall start from the formula of Hoorweg-Weiss ($i = \frac{a}{t} + b$). Let us make $i = 2b$, then $t = Chr$ (chronaxy).

$$2b = \frac{a}{Chr} + b; \quad Chr = \frac{a}{b}. \tag{9}$$

In other words chronaxy equals the ratio of short-term threshold to the protracted one.

If chronaxy increases, according to generally accepted opinion it implies a decrease in rapidity of reaction of the tissue. Obviously this decrease should be related to an increase in threshold time of excitation.
296 Let us see now what controls the increase in chronaxy.

From the equation $Chr = \frac{a}{b}$ it follows that chronaxy may increase due to the following reasons:

1) If a increases while b remains constant;
2) If a does not change while b decreases;
3) If a and b both increase but a increases faster than b;
4) If a and b both decrease, a more slowly than b.

All these cases are possible since changes in a and b, as has been shown above, may take place in either direction*.

From the above it is clear that chronaxy cannot serve as a measure of reaction speed, since its increase may take place with increase as well as decrease of excitation time necessary to cause a reaction. It cannot serve as a measure of excitability, since its increase may be observed b both on increase and decrease of excitability thresholds a and b.

Moreover, a case is possible where a and b increase or decrease proportionately to each other. Chronaxy will then remain constant in spite of decrease in protracted excitability and increase in threshold excitation time.

What measure is this then of speed of reaction?

This has originated from the fact that chronaxy is a ratio between two independently changing values (a and b), each of which expresses quite a definite property of the tissue.

That changes in chronaxy do not correspond to changes in threshold-excitation time and excitability can also be seen from the logarithmic intensity-time curves. In order to estimate chronaxy from such curves, it is necessary to draw a line above the level of the logarithm of the rheobase, at a distance of logarithm 2 (lg 2 = 0.3), up to the intersection with the curve, and to drop a perpendicular from this point to the abscissa, which will determine the logarithm of chronaxy (Figure 162, A). In Figure 162, B, the value of a, i.e., the threshold time of excitation, remains unchanged while the rheobase (b) increases. As a result, a decrease in chronaxy takes place which should supposedly indicate an increase in speed of reaction which, in fact, is not so.

In Figure 162, C, only a increases, while b and n remain unchanged. Chronaxy increases as well. This is the only case where chronaxy may serve as an accurate measure of the threshold time of excitation.

Indeed, if the rheobase in the formula $Chr = \frac{a}{b}$ does not change, then chronaxy becomes a value proportional to a and may serve as a measure of this value.

297

Examples of Errors in Chronaximetry

We shall give a number of specific examples of the lack of correspondence between chronaxy changes and those of the time necessary to cause excitation in the tissues.

Example 1. Let us consider Figure 168 which shows voltage-time curves of a nerve subjected to 5% ethyl alcohol vapor. The lower curve (1) corresponds to the condition of the nerve, before narcosis. Its rheobase

---

* If we take the more general formula: $i = \frac{a}{t^n} + b$, then chronaxy will be determined by the following equation: $Chr = (\frac{a}{b})^{\frac{1}{n}}$. It is not difficult to see that all arguments regarding the effect of the change of values a and b upon chronaxy are also applicable to this more general formula.

is 0.059 volts (Table 51), while the short-term threshold of excitability (a) is 12 millivolts milliseconds. The nerve was subjected to the action of narcotic vapor. After 38 minutes the curve shifted to position 2, while after 1 hour 36 minutes it occupied position 3.

Accordingly, its excitability constants also increased. The rheobase (b) increased to 0.98, i.e., 17-fold, while the short-term excitability threshold (a) increased somewhat less, to 73 millivolts milliseconds, i.e., 6-fold. In a word, a marked decrease in excitability occurred, and consequently the threshold time of excitation increased. As to chronaxy, (Table 51), it will be seen that not only did it not increase as expected (if it really were a measure of speed of reaction), but rather decreased from 0.5 to 0.32 milliseconds. This is quite correct, since on the right hand side of the formula $Chr = \frac{a}{b}$ b increased more than a.

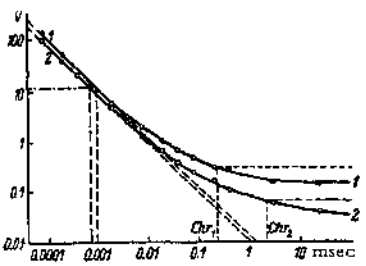


FIGURE 170. Effect of 10% ethyl alcohol on excitability of a frog nerve-muscle preparation (according to Nasonov and Rozental', 1953)

Logarithmic voltage-time curves: 1—before narcosis; 2—same preparation 45 minutes after emerging from a state of full narcosis; n = 0.96.

Example 2. The voltage-time threshold of a frog nerve was measured (Figure 170, 1), after being treated by 10% alcohol vapor to cause complete loss of ability to conduct impulses. Subsequently, the action of narcotic was stopped and after 45 minutes, a curve was again drawn (Figure 170, 2). These curves show clearly that everywhere there was an increase in excitability and a decrease in threshold time of excitation. No matter what voltage was used, a larger threshold-time of action was necessary to obtain excitation before the onset of narcosis than after it. Table 53 shows that the rheobase (b) decreased from 0.14 to 0.03 volts, i.e., 4.5-fold. The short-term threshold of excitability (a) decreased from 11 to 8.7 millivolts milliseconds, i.e., 1.26-fold, consequently the threshold time of excitation shortened to the same extent. Using the picturesque expression of Lapicque, the tissue became "more rapid". Obviously, if chronaxy really characterized the "velocity" of the tissue, it should also shorten; however, the data in Table 53 indicate that it increased 9-fold.

298 At the same time all this is not accidental but quite logical, since in the right hand part of the equation $Chr = \frac{a}{b}$ the denominator (b) decreases more markedly than the numerator (a).

Example 3. Change in voltage-time curves of a rat nerve on cooling (Figure 171). The rheobase and excitability thresholds increased along the whole length of the curve, while chronaxy remained unchanged. In terms of chronaximetry this means that on cooling the nerve from 20 to 5°C its excitability decreased, while the velocity of reaction remained the same. However, the error of this statement is clear. As in the previous case, using any voltage higher than the rheobase, the threshold time necessary for appearance of excitation on cooling of the nerve increased,

and did not remain unchanged. In this case too, the method of chronaximetry led to a gross error. The same is also observed on cooling a pigeon nerve (Figure 189, A).

Table 53

Effect of 10% ethyl alcohol vapor on excitability constants of a frog nerve, and on its chronaxy (according to (Nasonov and Rozental', 1953)

Constant	Before narcosis (see Figure 170, 1)	Forty-five min after cessation of narcosis (Figure 170, 2)
Increment of curve	0.96	0.96
Protracted threshold of excitability (in volts)	0.14	0.03
Short-term excitability threshold (in mvolt·msec)	11.0	8.7
Chronaxy (in msec)	0.25	2.30

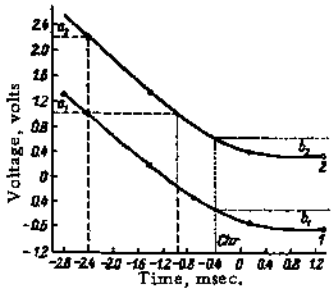


FIGURE 171. Logarithmic voltage-time curves on cooling a rat nerve (according to Nasonov and Suzdal'skaya, 1956b)

1—at 20°C; 2—at 5°C. The remaining legend as in Figure 162.

The source of error is clear. As shown in Figure 171, the excitability in all segments of the curve of voltage-time changed similarly. Consequently, in the right hand part of the equation $Chr = \frac{a}{b}$ both the numerator and denominator decreased to the same extent, as a result of which chronaxy did not change*.

Example 4. One more example will be given, similar to the cases in which chronaximetry is used in medicine, i.e., the change of excitability of the peripheral segment of a cut rat nerve. The consecutive excitability curves of such dying segments are shown in Figure 172, from which it will be seen that the rheobase increased continuously while chronaxy decreased. It might be concluded that in this case, too, the excitability threshold increased before dying, and the velocity of reaction also increased (a physician might interpret this as a favorable sign of a sort). However, here too, the method of chronaximetry leads to error, since in reality the threshold time of excitation increases continuously, as would be expected in a dying tissue.

---

* We gave an example of cooling of a rat nerve during which chronaxy did not change. However, in other particular cases chronaxy might increase somewhat and sometimes it might even be shortened. All this depends on the ratio between speeds of increase of a and b.

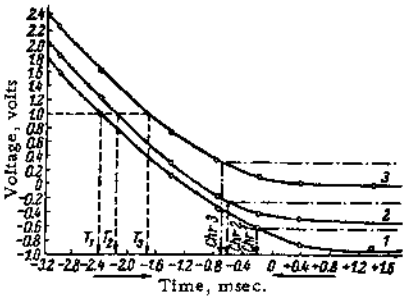


FIGURE 172. Logarithmic voltage-time curves of peripheral segment of rat nerve, at various time intervals from beginning of the experiment; (according to Nasonov and Suzdal'skaya, 1956b)

1—immediately after cutting;
2—90 minutes after cutting;
3—240 minutes after cutting.
The arrows indicate changes in chronaxy (Chr) and in threshold time of excitation (T).

Similar results were obtained by Apostolaki and Deriand (1925) in whose experiments a peripheral segment of a frog nerve showed an unchanging chronaxy for 8 to 10 days after cutting, accompanied by progressive fall in the rheobase.

The reason for the decrease in chronaxy on increasing the threshold intensity and the time of excitation was that b increased at a faster rate than a.

If in the right hand part of the equation $Chr = \frac{a}{b}$, b changes relatively less than a, then chronaxy will change in the same direction as will constant a, i.e., it will give qualitatively correct indications of the change of threshold time of excitation. Quantitatively, however, these data will be distorted by changes in a. It has been mentioned that chronaxy accurately reflects changes in threshold time of excitation only where the denominator, i.e., the rheobase (b) in the equation $Chr = \frac{a}{b}$ remained unchanged, while the numerator (a) changed. However, such cases are the exception rather than the rule.

Errors due to chronaximetry, taken from data in the literature

In the majority of studies in which changes in excitability were investigated, only chronaxy and rheobase were given. The threshold of short-term excitability was not investigated, and consequently the threshold time of excitation was also not studied. However, in certain particular cases
300 where n = 1 these values may be found from chronaxy and the rheobase, since from the equation $Chr = \frac{a}{b}$ it follows that a = bChr*.

---

* Renquist, Leskinen and Parviainen (1931), studying the effect of urethane on frog muscle, showed that at the beginning of narcotization the rheobase increased, while chronaxy at first decreased and subsequently increased. The product of these values did not change, or increased slightly with the rheobase. The authors designated this product by the letter a. They called it the hyperbolic parameter, or the second constant of the hyperbola, not realizing that this value was nothing but the threshold strength of excitation, by stimuli of short duration.

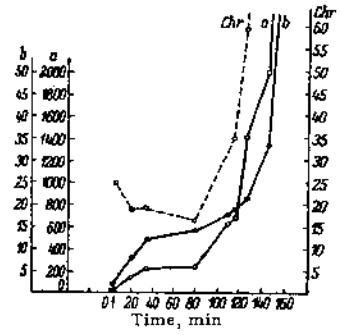


FIGURE 173. Changes in rheobase (b), chronaxy (Chr) and threshold of short-term excitability (a) after the action of phenol on a frog nerve (according to Magnitskii and Muzheev, 1930, from Nasonov and Rozental', 1953)
The arrow marks onset of narcosis.

Since many studies were performed on vertebrate nerve or muscles where n is always close to 1, the value of a may be reproduced and its dynamics compared with that of b and Chr.

The work of Magnitskii and Muzheev (1930) will be taken as an example. Here the changes in rheobase and chronaxy of a frog nerve under the influence of phenol and cocaine were studied. The authors assumed that the rheobase is an expression of the nerve's excitability, while chronaxy expressed the lability or functional mobility of the nerve (according to the terminology of N. E. Vvedenskii). According to their data, the rheobase of the nerve increased continuously under the influence of narcotics, while chronaxy at first decreased and subsequently increased (Figure 173). On this basis the authors concluded that "parabiosis is associated with decreased excitability. The functional mobility increases at the beginning and decreases later. The characteristic signs of parabiosis correspond exactly to the period of increased functional mobility" (Magnitskii and Muzheev, 1930, pp. 81-82).

If now, on the basis of these data, the value of a is calculated, it is possible to see that the short-term threshold of excitability, and therefore also the threshold time of excitation increase continuously (Figure 173), at first forming a plateau like the rheobase, and then decreasing rapidly with the death of the tissue. The biphasic nature of the change in chronaxy resulted from the fact that simultaneous increase in a and b was not always strictly proportional. Of course we are very far from denying altogether the biphasic nature of the change in excitability during narcosis. It undoubtedly exists and may be detected by measuring the constants a and b, but not by measuring chronaxy.

In Soviet and foreign literature there are many papers claiming that at the onset of narcosis the rheobase increases while chronaxy decreases. A paradoxical conclusion is drawn from this fact that decrease in excitability of a tissue may supposedly be accompanied by an increase in speed of re-
301 action, and those authors even ascribe a special meaning to this fact, e.g., Schriever and Ehrhardt (1939).

However, the determination of the value of a from the chronaxy and rheobase, given in this paper, shows that here, too, a false conclusion was drawn, based on the conviction that chronaxy is a measure of the speed of reaction.

Two more examples will be given from clinical practice obtained during the study of limb-muscle excitability in normal cases and during various disease processes*. In various diseases the general decrease in muscle

---

* The measurements were performed by D. L. Rozental' and V. N. Filippova at the State Institute of Traumatology, Leningrad, 1954.

excitability is often accompanied by lengthening of chronaxy, due to the fact that the constant a increases faster than b. Thus the lengthening of chronaxy is an index of deterioration in the condition of the organ.

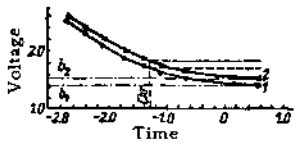


FIGURE 174. Logarithmic voltage-time curves of normal and pathological human muscles of the hamstring group (according to Rozental' and Filippova, 1957)

1—healthy lower extremity: a—529 millivolts milliseconds; b—24 volts, Chr—0.68 milliseconds; 2—diseased lower extremity (ankylosis of the right knee joint): a—696 millivolts milliseconds; b—32 volt, Chr—0.61 millisecond.

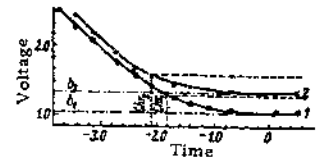


FIGURE 175. Logarithmic voltage-time curves of normal and pathological human muscles (m. biceps brachii) (according to Rozental' and Filippova, 1957)

1—healthy upper extremity: a—80 millivolts milliseconds, b—7 volts, Chr—0.016 milliseconds; 2—a diseased upper extremity (residual phenomena after spastic paralysis): a—127 millivolts milliseconds, b—16 volts, Chr—0.008 milliseconds.

But there are other cases, too. Figure 174 shows 2 voltage-time curves of the hamstring group of muscles: curve 1—normal, curve 2—during pathological state, in one and the same person. The threshold intensity and threshold time of excitation of the affected limb are seen to be increased along the whole curve, while chronaxy remained unchanged. Consequently chronaxy, in spite of appearances, here indicated the lack of change in excitability. The reason for this was the fact that the constants a and b changed proportionately.

Another case is illustrated in Figure 175. Curve 1 corresponds to the excitability of the biceps muscle of a healthy human arm, and curve 2 corresponds to that of a diseased arm (residual phenomena after spastic paralysis). Here, with general decrease in excitability and increase in threshold time of excitation, shortening of chronaxy is observed, which may also lead the physician to the false conclusion that the diseased muscle reacts more rapidly to stimulation than the healthy one. The reason for shortening of chronaxy in this case is clear. The constant b in this case increased sooner than a. Unfortunately, such cases often remain unpublished, since they conflict with the generally accepted meaning of chronaxy.

These examples of errors due to the use of chronaximetry have been given because this method has become so deeply rooted in medical and physiological practice. The number of examples may be multiplied, but those given are sufficient to refute the concept of chronaxy as a time factor.

302

Certain Disputable Questions

After the publication of our studies on the time factor in tissue excitability (Nasonov and Rozental', 1953), the editorial board of "The Physiological Journal of the USSR" announced a symposium on this theme. As the result of this a number of articles appeared in this journal, the authors of which took issue with us and in which our replies are also included (Uflyand, 1954; Abrikosov and Darkshevich, 1954; Kiselev, 1954; Navakatikyan, 1954; Nasonov and Rozental', 1955). In addition, discussions were held on this subject by the Moscow (1953) and the Leningrad (1954) Societies of Physiologists, Biochemists and Pharmocologists.

Without discussing matters of secondary importance, and noting a number of objections based on misunderstandings, we will deal only with those questions which we consider to be to the point in this issue.

The first objection was directed to the method of determining the constant a and was related to studies on the subject of very short, nonphysiological time intervals of excitation; it also implied that excessive, nonphysiological voltages might lead to tissue damage and to distortion of the results. As opposed to that, chronaxy supposedly corresponds to excitation intervals in the middle part of the intensity-time curve, in the region close to optimal physiological intervals of current where a comparatively small voltage acted on the tissue.

However, our opponents evidently did not take into account the fact that the proposed method of estimating the value of a is based on the finding of threshold voltage at a current of a given duration. As in other cases, there is a liminal voltage which barely causes a response reaction in the most sensitive fibers. Obviously no damage whatsoever occurred, because the high voltage of the passing current is compensated by its short duration. This is the biological meaning of the law of inverse proportions between the intensity of excitatory current and the duration of its action on living protoplasm.

As far as determination of chronaxy is concerned, this is associated with much less adequate effects since its measurement is based on determination of the rheobase. This was usually revealed simply by pressing the button, a procedure which took not less than 0.1 second. If it is assumed, as is usual in the case of frog muscle, that the duration of the rising path of the excitation wave of the nerve is 0.1 millisecond, the time of passage of direct current through the nerve during determination of the rheobase is 1,000 times greater than that required! In addition, the method of pressing the button does not ensure discontinuation of the current after appearance of excitation. Above-threshold current continued to pass for a long time throught the fiber after excitation had propagated along the nerve. We rejected the determination of rheobase by a pulse of direct current of undetermined duration, and we found it by the use of a condenser discharge of high capacity.

303 For the determination of constant 'a' on a frog nerve a period of 0.001 millisecond was used, i.e., within time limits differing only 100-fold from physiological ones.

Another basic objection is that chronaxy, whatever it may mean, has the advantage that its value supposedly does not depend on the method of determination, while the absolute value of constant 'a' depends on the method by which it was measured and on the histological structure of the tissue.

Originally Lapicque and his co-workers assumed that the value of chronaxy does not depend on "instrumental conditions" (Lapicque's expression) or on the anatomic structure of the organs; while both constants in Hoorweg's formula change proportionately on changing the resistance of the chain or the shunt. If this were so, chronaxy would be an absolute characteristic of the tissue.

Even now many investigators believe in this "absoluteness" and they assume that on the basis of chronaxy the action of different human muscles may be compared, in spite of the different conditions of passage of electric current through the tissue.

However, Lapicque in his book (1926, p.233) admits that the interelectrode distance affects the value of chronaxy. This has been shown by his co-workers Cardot and Laugier (1914) on frog nerve, where by changing the distance between electrodes from 2 to 9 mm, chronaxy may be increased by 100%, while the rheobase drops by 25%.

At a later date, Jinnaka and Azuma (1922) while studying the sartorius muscle of the frog found that chronaxy measured by means of capillary electrodes is smaller than if large electrodes were used. Davis (1923) confirmed their observations and he is of the opinion that these facts are a serious obstacle to chronaxic specificity of various muscles. According to his data obtained with the same tissues, chronaxy measured by means of a capillary electrode of a diameter of 3-75 μ, or by contact with a metallic thread, equals 0.2-0.5 milliseconds, while the large electrodes of Lucas show a value of chronaxy of up to 20 milliseconds, i.e., of 100-fold difference! Varying the dimensions of the electric electrode, all intermediate values between these figures may be obtained at will.

The above-mentioned obstacles to comparisons of activity according to chronaxy could be overcome by standardizing the conditions of the experiment, and by always observing an appropriate interelectrode distance and electrode dimensions. However, in determination of chronaxy in man by applying electrodes to the skin, the obstacles encountered become unsurmountable. Under these conditions the actual interelectrode distance, measured by the point of entry and exit of the force lines from the nerve or the muscle, and the area of exit corresponding to the dimensions of the electrodes, is indefinable.

Chronaxy of such organisms as spirogyra on the one hand, and nerves and muscles on the other, cannot be unconditionally compared, as was done by Lapicque (1926), since both the interelectrode distance and the diameter of the electrodes differ quite considerably from each other.

It is well known that simple increase in serial resistance in the chain does not at all affect the value of chronaxy on direct application of the electrodes to the nerve or muscle, while it markedly distorts chronaxy when
304 it is determined through the skin in human beings. The latter fact is probably explained by the complicating effect of polarization on the skin surface.

All these facts completely destroy the myth of absolute value of chronaxy, and they show that comparison of various tissues by this means is relative and approximate only.

Thus, on a theoretical basis, following the analysis of Hoorweg's formula, and also on the basis of specific examples taken from the literature and from our own experiments, we concluded that chronaxy is not a measure of the stimulation time necessary for appearance of excitation in conducting tissue. This concept had become deeply rooted due to the numerous studies of Lapicque and his co-workers. Many representatives of Vvedenskii's school were of the opinion that chronaxy is an approximate measure of lability or functional mobility of tissues.

In our opinion, all these views on chronaxy are incorrect.

One of the reasons for this erroneous concepts is that on comparing the excitability of various tissues, the more slowly reacting tissues such as heart muscle and smooth muscles of vertebrates, muscles and nerves of certain mollusks, chloroplasts of algae, etc. possess a higher chronaxy than the rapidly reacting skeletal muscles and nerves of vertebrates. This coincidence between reaction-rapidity series and that of reciprocal chronaxy served Lapicque as one of the basic arguments for treating chronaxy as a time-characteristic of tissues. In a sufficiently rough approximation this coincidence really does take place, depending on the fact that on comparison of various tissues with each other, the rheobase (b) changes many times less than does the short-term threshold of excitability (a), and therefore, the threshold time of excitation. This was pointed out when we discussed the possibility of comparing the excitability of various tissues (page 294). Under conditions where b changes less than a, chronaxy value changes more or less proportionately to 'a' and becomes an approximate measure of the threshold time of excitation. However, as pointed out, tissues of similar excitability may differ in chronaxy and in the threshold time of excitation (Figure 169, 3, 4). In cases where the action of these or other agents on the same objects is studied, 'b' very often varies more than 'a'. Under such conditions, of course, coincidence between the changes of these values and chronaxy is impossible since the latter is markedly affected by the rheobase.

The concept of chronaxy as a measure of the threshold time of excitation is an erroneous one, and lies in the fact that in the experiment, at first the rheobase is measured, and multiplying it by 2 one obtains the chronaxy. Subsequently, due to the action of various agents, it is not this rheobase which is used for the determination of alteration in chronaxy but another rheobase already altered under the influence of the
305 agent. In other words, for the measurement one uses a "rubber arshin"* which either stretches or shortens unpredictably in the process of measurement. This has been pointed out by Beritov in his book (1947).

The question arises: what is chronaxy? Does it at all represent any property of the tissues, or should it be regarded as an artifact of no real significance?

In the literature dealing with chronaxy it has been repeatedly pointed out that if the formula of Hoorweg-Weiss ($i = \frac{a}{t} + b$) is taken, where the power exponent of t equals unity ($n = 1$) chronaxy may be interpreted as a value proportional to the minimal threshold-energy necessary for excitation.

---

* [Arshin-Russian measurement of length ~ 28 inches].

However, this equation, which holds for the particular case where $n = 1$, has no meaning if it is remembered that the value of n in different organisms varies from 0.5 to 1.0. In the case of $n = 0.5$ (Nernst), no minimal energy exists at all, but chronaxy exists all the same (Cremer, 1929, p.249).

Thus, this interpretation of chronaxy should also be regarded as erroneous.

However, it would be quite unjustified to consider chronaxy as an artificial value. It no doubt reflects one property of tissues related to their excitability, which may be represented mathematically: when $i = \frac{a}{t^n} + b$, $Chr = (\frac{a}{b})^{\frac{1}{n}}$*. On a logarithmic graph chronaxy coincides exactly with the point of intersection of the two asymptotes (Figure 162). This may be shown algebraically. The equation on the left hand asymptote will be $\log i = \log a - n \log t$, while the equation of the right hand asymptote will be $\log i = \log b$. Consequently, the point of their intersection will be determined by the equation $\log b = \log a - n \log t$, or $n \log t = \log \frac{a}{b}$, or $t = \frac{a}{b}^{\frac{1}{n}}$, the latter being the equation of chronaxy.

Figure 162, D shows that no matter what the slope of the left hand asymptote of the logarithmic graph is, chronaxy should always coincide with the intersection of the asymptotes. Two rules, which at a sufficient distance from the point of intersection may be approximately expressed by the formulas $i = \frac{a}{t^n}$ and $i = b$ clash here.

In this sense chronaxy describes with mathematical accuracy for any tissue, the region where the time of action of the stimulus begins to affect the appearance of excitation to some extent. In this sense chronaxy may be considered as the strictest and most accurate method of evaluation of that quantity the determination of which is stated by the so-called "effective time"**. This was pointed out by Lapicque (1926, p. 225).

306 This is all which may be claimed for chronaxy. The latter may measure the time of excitation and excitability only when the rheobase remains unchanged, which practically never happens. The ruling concept that chronaxy is a constant which determines the threshold time of excitation or the speed of reaction of the tissue may be considered only as a deeply-rooted misunderstanding by physiologists. As a result, in the 60 years which have passed since the work of Hoorweg and Weiss, no physiologists studied properly the constant 'a', which really characterizes the threshold time of excitation and excitability after short-term stimuli. Instead, only chronaxy and rheobase were investigated. The conclusions arrived at by the use of these two indexes require a most serious revision. In the

---

* When $n = 1$ $Chr = \frac{a}{b}$.

** "Effective time" is usually determined as that time interval starting from which the appearance of excitation no longer depends on the time of passage of the stimulation current. However, according to the formula of Hoorweg, such a time does not exist, since the intensity-time curve approaches asymptotically the level of the rheobase (see Figure 161). Therefore the usual practical determination of the "effective time" was always arbitrary, and wholly dependent on the sensitivity of the method used. Increasing the sensitivity may shift the "effective time" to the region of greater intervals, as required.

course of such a revision the following chapters state the results of the investigation of two problems: change in excitability on sectioning a nerve, and effect of temperature on excitability.

Chapter 4. Change in Excitability on Sectioning a Nerve

Historical Aspects of the Problem*

The problem of increased excitability of a sectioned nerve was discussed in the last century by Sechenov (1861), who assumed that the reason for this increase was a molecular change in the protein substrate of the nerve fiber, arising in the process of cutting it ("a certain vibration of the nerve particles"). Later, M. Lapicque (1923) observed that on isolation of a frog nerve from its central connections by cutting it, a considerable decrease in the rheobase (increase in excitability) occurred with a simultaneous 2-fold increase in the nerve's chronaxy. This observation was later confirmed by many authors. The phenomenon itself was treated by L. Lapicque as a disturbance of "subordination", since it was assumed that it was dependent on the cessation of some constant influence of the central nervous system on the peripheral components (Lapicque, 1936).

Starting from the classical concept of chronaxy as a measure of rapidity of reaction, the paradoxical conclusion may be drawn that on cutting a nerve its excitability increased, while the speed of reaction decreased. This paradox was made even more obvious by the data of Monnier and Jasper (1932), according to which cutting of a frog nerve really increased chronaxy values by 33%; while the speed of impulse conduction which should have correspondingly decreased, increased by 22%**.

Studies of excitability of a nerve of a warm-blooded animal separated from central connections gave inconclusive results (see Uflyand, 1938). Thus, Lambert, Skinner and Forbes (1933), on cutting nerves of cats, ob-
307 served a temporary increase in excitability and prolongation of chronaxy only at a short distance from the lesion. From this it may be concluded that the reason for changes in excitability observed was the nerve cutting procedure.

Change in excitability of rabbit nerve after separation of the latter from the central sections was later investigated by Vul and Konikov (1937). According to their data, cutting of the nerve caused insignificant changes in chronaxy (2-10%), while the rheobase shifts sometimes coincided with the shifts in chronaxy, but at other times did not. The authors assumed that the observed changes may be explained by factors originating from the wound field.

The above-mentioned studies of American and Soviet authors do not conform to the ideas on subordinational influence of the centers on the

---

* We shall quote the extensive literature on so-called "subordination". We shall limit ourselves only to the more interesting studies. A review of the literature on this problem may be found in papers by Davis and Forbes (1936), Uflyand (1938), Magnitskii (1948) and Golikov (1950).

** Later, Monnier (1934) was forced to admit that in the given case chronaxy was not a time factor.

periphery, as Lapicque understood them. These contradictory data induced us to revise the problem of changed excitability on cutting the nerve, in the light of analyses stated in the previous chapter (Nasonov and Rozental', 1956).

Materials and Methods

Studies were performed on a grass frog and a white rat. Excitability was determined by the use of the instrument described earlier (p. 279).

The frog was fixed on its ventral surface on a cork plate by the use of a wet bandage. Many pins were stuck into the cork so that the animal was immobilized between them, while normal blood circulation was entirely preserved. In the upper part of the thigh a segment of the sciatic nerve was exposed for 2 cm (without narcosis) and silver electrodes were introduced beneath it at a distance of approximately 5 mm from each other. The appearance of impulses was detected by movements of the hind limb toes.

The rat was placed prone on a wooden board, and fixed by adhesive tape on top of the spread legs, back, tail and head. If the animal was narcotized by ether vapor, the sciatic nerve was exposed in the region of the upper part of the thigh for approximately 2 cm and silver electrodes were introduced underneath it. After complete awakening of the rat from narcosis, excitability of the nerve was determined by movement of the hind toes. During the experiment the nerve was moistened with Ringer's solution. The nerves of the rat and frog were separated from the CNS by constriction with a thread loop, previously introduced proximal to the electrode. In certain cases the cervical spine was cut.

Experiments with Frog Nerve

The experiments were performed in January 1952 in the following manner. At first, measurements of two values were taken at 5-minute intervals: 1) the protracted threshold of excitability (constant b), which with our instrument was determined by the minimal number of volts necessary for excitation to take place after a condenser discharge of a 90 microfarad capacity; 2) the short-term threshold of excitability, or the constant a, in absolute units. To illustrate this by a concrete example:

Let us choose a time interval of 0.00004 milliseconds. In order to obtain a threshold excitation of a frog nerve in this action time, a voltage of 140 volt was required (or 140,000 millivolts). Subsequently, we measured
308 the protracted threshold of excitability on the same tissue. The latter equalled 0.3 volts. It followed that, working in the region of short intervals, the formula $a = vt$ may be used for determination of constant 'a', instead of the formula $a = (v-b)\,t$. The error will not exceed 0.2%. This accuracy is more than sufficient.

Substituting in formula $a = vt$, the values of v and t thus obtained gives: $a = 140{,}000 \times 0.00004 = 5.6$ millivolts milliseconds. In practice, we used the same time interval—0.00004 millisecond for determination of 'a', in all the experiments.

After ensuring that measurements of the values of 'a' and 'b' at 5-minute intervals gave identical values, the nerve was separated from the central connections and the value of the constants was again determined immediately. Since chronaxy equals the ratio a:b, for each experiment the change in chronaxy could be calculated by these constants after tying the nerve*.

Table 54 shows the results of these experiments. It will be seen that isolation of the nerve from the center caused a considerable decrease in rheobase (by 60%) and an increase in chronaxy (by 93%), so that in this respect the earlier data of Lapicque (1923) and other investigators are completely confirmed.

Further it will be seen that the mean square error is many times less than the result obtained (Table 54), which is thus statistically significant. Also the result depended only to a small extent on whether the nerve was
309 separated by tying or by cutting the spine. Finally, not only did the rheobase decrease after separation (60%), but also the threshold of short-term excitability decreased, although to a considerably lesser extent (32%). As already stated, the coefficient 'a' was directly proportional to the threshold time of excitation. Its decrease probably indicated that the speed of reaction of the nerve increased (by 32%) after its isolation, in contrast to the almost 2-fold increase in chronaxy.

Table 54

Changes in the rheobase (b), the short-term threshold of excitability (a), and chronaxy (Chr) on sectioning a frog nerve (according to Nasonov and Rozental', 1956)**.

Method of separation	Initial value of b (in volts)	Change in b (in % of initial value)	Initial value of a (in mvolt·msec)	Change in a	Change in Chr
				% of initial value	
Tying of the nerve	0.36	−78.0	5.6	−59.5	+ 82.0
	0.24	−66.6	4.16	−23.0	130.0
	0.32	−62.4	5.12	−12.5	+132.0
	0.24	−66.7	4.72	−30.5	+104.0
	0.38	−36.8	5.9	− 2.5	+ 54.0
	0.18	−66.7	4.96	−54.8	+ 35.5
	0.38	−79.0	5.55	− 4.0	+ 39.5
Severance of spinal cord	0.16	−62.5	3.6	−40	+ 60.0
	0.36	−50.0	5.6	−50	0
	0.16	−31.0	3.84	−23	+ 11.5
	0.22	−63.6	6.4	−56.3	+ 20.0
Arithmetic mean	0.273	−60.3±4.6	5.04	−31.6±6.4	+ 60.8

---

* This method of determination of chronaxy is an approximate one. It can be very accurate only for the canonic curve of Hoorweg.

** In these and subsequent experiments the coefficient n did not change after isolation of the nerve from the CNS. This is clearly seen in Figure 176, where the slope of the logarithmic curve remains unchanged before and after tying the nerve.

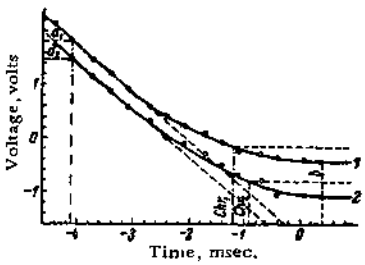


FIGURE 176. Logarithmic voltage-time curves of a frog nerve (according to Nasonov and Rozental', 1956)

1--nerve not separated from centers; 2--same nerve immediately after tying. Remaining legend as in Figure 162.

Following is another example of the gross errors made in the use of chronaximetry if chronaxy is considered as a measure of reaction-speed. Let us analyze Figure 176. Obviously, the excitability increased considerably. It is also clear that no matter what intensity was used to excite the nerve, the threshold time of excitation decreased on tying the nerve. It should be pointed out that chronaxy increased considerably, which leads to the contradictory conclusion that the reaction speed decreased. In the given case the reason for the increase in chronaxy is quite clear: the constant 'b' decreased more than constant 'a'. That is why according to the data of Monnier and Jasper (1932), the rate of conduction of the impulse increased under these conditions in spite of increased chronaxy.

Experiments with Rat Nerve

Table 55 shows the data obtained on tying a rat nerve. The rheobase was determined at 90 microfarads, and the short-term threshold of excitability determined (a) just before tying the nerve and (b) immediately afterwards.

From the figures in Table 55 it is apparent that the effect of separation of the rat nerve from the central connections is very similar to the effect obtained in the frog. A marked decrease in rheobase and a somewhat lesser decrease in the short-term threshold of excitability may be noted, which indicate increased excitability and decreased threshold time of excitation. At the same time chronaxy was found to be considerably increased, which, in terms of the classic concept of chronaxy as a measure of reaction time, should be associated with increased threshold time of excitation. Here, as in the case of the frog nerve, faulty concepts of the meaning of chronaxy result in erroneous interpretations.

The question was then raised: Is the observed effect of increased excitability and decreased threshold-time of excitation in any way related to isolation from the central nervous system, as was assumed by L. Lapicque (1936) and many others?

310 In order to overcome this difficulty, the cervical spine was cut in the following series of experiments with rats, instead of tying the nerve. The results are shown in Table 56.

These results (Table 56) show that even if decapitation leads to some changes, they are so slight that they are limited to the range of experimental error. Consequently, no subordinational influences arise from the brain.

311 This conclusion is further strengthened by the following series of experiments in which the rats were first decapitated, and the nerve then tied at a distance of 1 cm above the proximal electrode. The results of these

experiments are shown in Table 57, from which it follows that tying the nerve of a decapitated rat gives exactly the same effect as that obtained in a nondecapitated animal (compare Tables 55 and 57). A considerable decrease in rheobase (62.8% on the average) is observed here, a somewhat less distinct fall in threshold of short-term excitation (41.6% on the average), and an increase in chronaxy (69.6% on the average).

Table 55

Change in rheobase (b), in the short-term threshold of excitability (a) and in chronaxy (Chr), on severing of rat nerve (according to Nasonov and Rozental', 1956)

Initial value of b (in volts)	Change in b (in % of initial value)	Initial value of a (in mvolt·msec)	Change in a	Change in Chr
			% of initial value	
0.1	−60	5.76	−50.0	+25
0.08	−50	5.2	− 6.5	+87
0.1	−40	6.8	−29.5	+76
0.04	−50	3.6	−11.0	+78
0.08	−50	4.65	−33.0	+34
0.1	−60	5.2	−32.0	+69
0.08	−25	4.56	−34.0	−12
0.04	−50	3.28	−27.0	+46
Arithmetic mean				
0.0775	−48 ± 4	4.9	−28 ± 5.4	+50.3

Table 56

Effect of decapitation on excitability of rat nerve (according to Nasonov and Rozental', 1956)

Initial value of b (in volts)	Change in b (in % of initial value)	Initial value of a (in mvolt·msec)	Change in a (in % of initial value)
0.1	−40.0	5.85	−50.7
0.16	−12.5	7.2	−16.5
0.12	0	4.0	+ 4.0
0.1	−20.0	4.25	− 6.5
0.06	+66.5	2.4	−33.0
0.16	−12.5	4.3	− 8.0
0.16	0	4.8	0
0.1	0	3.52	+ 2.0
0.1	−40.0	2.72	0
Arithmetic mean			
0.118	− 6.5	4.48	− 4.75

Table 57

Effect of tying the nerve at a distance of 1 cm from the electrode, in decapitated rats (according to Nasonov and Rozental', 1956)

Initial value of b (in volts)	Change in b (in % of initial value)	Initial value of a (in mvolt msec)	Change in a	Change in Chr
			% of initial value	
0.12	−66.6	4.15	−23.0	+130.0
0.08	−75.0	4.65	−65.4	+ 38.0
0.1	−80.0	3.2	−55.0	+124.5
0.1	−60.0	3.7	−43.5	+ 41.0
0.16	−75.0	4.8	−50.0	+100.0
0.04	−50.0	2.72	−29.5	+ 41.0
0.06	−33.0	2.56	−25.0	+ 12.5
	Arithmetic mean			
0.0943	−62.80 ± ± 6.26	3.7	−41.6 ± ± 6.1	+ 69.6

Table 58

Effect of tying the nerve at a distance of 2 cm from the electrode in decapitated rats (according to Nasonov and Rozental', 1956)

Initial value of b (in volts)	Change in b (in % of initial value	Initial value of a (in mvolt·msec)	Change in a (in % of initial value)
0.14	−57	4.0	− 4
0.16	0	4.8	0
0.10	−60	4.0	−32
0.06	0	2.56	0
	Arithmetic mean		
0.115	−29.3	3.84	− 9

312 When the nerve was tied somewhat further away from the electrode (2 cm), the described effect of increased excitability was considerably lower, as follows from Table 58*.

These experiments contradict the views of Lapicque, according to which the increased excitability of the nerve after its severance from the central connections depends on cessation of controlling influences constantly arriving from the central nervous system, which maintain the excitability of the nerve at a certain lower level. If this were true then the increased

---

* This fact may be explained in that at a close distance of the electrodes from the site at which the nerve was tied, subthreshold impulses, spreading with decrement, may also reach the electrodes in addition to the ordinary impulses.

excitability after tying the nerve should persist for longer periods at the new level. In reality this is not so. Measurement of excitability of the nerve at longer time intervals after its isolation showed that the increase in excitability was very transient, and that after 30 to 40 minutes it again returned to initial levels (Figure 177).

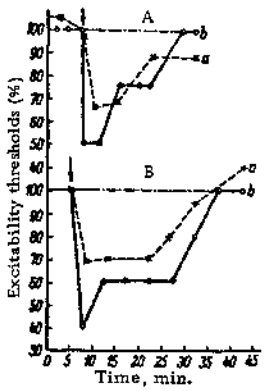


FIGURE 177. Change in threshold of the short-term (a) and protracted (b) excitability of frog nerve after isolation from central connections (according to Nasonov and Rozental', 1956)

A, B—2 different experiments. The initial level of excitability is taken as 100%. The arrow marks the time of tying the nerve.

What then is the real reason for the increase in excitability?

The theory of Vul and Konikov (1937) that excitability of the nerve temporarily increased due to multiple nerve impulse shocks coming from the wound area seems to us most probable. That such multiple nerve impulses really take place is apparent from the convulsive movements of the hind toes which are well marked for several seconds after tying the nerve.

However, could the impulses propagating along the nerve raise its excitability even temporarily?

In order to solve this problem the following series of experiments were carried out. The sciatic nerve of a rat was exposed for a length of 2 cm, by the above-described method. A pair of electrodes was introduced beneath it to determine excitability. A second pair of electrodes was placed somewhat higher, to excite the nerve by a current from an induction coil of DuBois-Reymond with a Bernshtein switch. Firstly, the ordinary excitability of the nerve was determined, and subsequently the nerve was stimulated for 10 seconds through the pair of additional electrodes at a distance of 25 cm from the coils, and with a frequency of 18 cps. The excitability of the nerve was again determined. The results of these experiments are shown in Table 59, from which it follows that the effect on the nerve of preliminary application of impulses for a short-time period closely resembles tying of the nerve (compare Tables 55 and 59).

313

Here, as on tying the nerve, the rheobase was markedly reduced, the threshold of short-term excitability somewhat less so, and chronaxy was elevated. Accordingly, not only the direction of shifts of excitability indexes, but also their absolute values, were very similar in both cases*.

Thus, both our experimental data and certain studies of previous investigators (Lambert, Skinner, Forbes (1933); Vul and Konikov (1937), make us think that Lapicque was wrong in assuming that the increased excitability of the nerve, when isolated from its central connections, is due to cessation of reaction of the nerve to the brain centers, which was denoted by Lapicque as "subordination". We assume that this effect is due to passage

---

* Golikov (1934), using an isolated frog nerve muscle preparation, described increased chronaxy and decreased rheobase after a marked excitation by induction current.

through the nerve of a series of impulses originating at the site of the surgical wound. In frogs this effect spreads from the wound not only along the nerve itself but also to the higher levels of the nervous system. In warm-blooded animals this effect is for some reason limited to shorter segments of the nervous chain.

Table 59

Effect of induction current on excitability of a rat nerve (according to Nasonov and Rozental', 1956)

Initial value of b (in volts)	Change in b (in % of initial value)	Initial value of a (in mvolt·msec)	Change in a	Change in Chr
			% of initial value	
0.22	−27.3	5.9	−16.0	+15.0
0.24	−41.6	6.1	−21.0	+35.5
0.24	−16.7	5.75	−14.6	+ 3.2
0.18	−55.5	4.8	−26.6	+64.5
0.22	−63.6	6.1	−47.4	+45.0
0.18	−44.5	5.6	−27.5	+28.6
0.22	−45.5	6.1	−18.5	+49.5
0.18	−22.2	5.6	−11.4	+14.0
Arithmetic mean				
0.21	−39.6 ± 6.5	5.74	−22.8 ± 2.4	+31.9

Do these inconclusive deductions pertaining to subordination minimize the role of the central nervous system as a regulatory and determining factor in the vital activities of the organism?

The answer is no. We have only shown that increased excitability after tying a nerve cannot be considered as a direct effect of cessation of subordination. This is particularly obvious in the case of rat nerves. However, it has also been shown that under experimental conditions the passage of impulses through the nerve may temporarily increase its excitability. This being so, there is firm basis for the assumption that in
314 the natural surroundings as well, impulses passing along the nerve from the centers may temporarily increase the excitability of the conductor. Such concepts of the mechanism of subordination were also expressed by other authors. It is highly probable that this mechanism is used by the organism for regulation of the level of excitability of the conducting pathways.

Chapter 5. Effect of Temperature on Excitability of Nerves and Muscles

Experiments with Nerves of Cold-Blooded Animals (Frogs, Tortoises)

One of the basic questions invariably arising from quantitative studies of one or another property of a living organism is that regarding the effect of temperature. The nature of effect of the temperature factor enables one to investigate this problem more fully. Tissue excitability is a temperature-determined property to some extent, and this property remains one of the most characteristic indexes of the functional state of conducting tissues. However, in spite of many studies on this subject, it still remains unclear how temperature may affect excitability.

In a number of earlier papers it was established that cooling may increase the excitability of nerves to a marked extent. But there are also indications that decrease in temperature lowers nerve excitability*. Gotch and MacDonald noted this contradiction between data in the literature and claimed that the reason for it was inherent in the fact that different authors used different methods of stimulation. According to their experiments, lowering of temperature increased excitability when the nerve was tested by galvanic current, and decreased it when induction current was used. Therefore, in discussing general increase in excitability, the experimental methods must be taken into account. Waller (1899) showed very convincingly that in the experiments of Gotch and MacDonald, the crux of the problem was the duration of current-action. With more prolonged impulses of current, decrease in temperature caused an increase in excitability. With short-lasting impulses the reverse was true. These data were later confirmed by L. and M. Lapicque (1907), by Lucas and Mines (1907-8) and by a number of other authors.

In more recent studies carried out by the chronaximetric method, there was also no uniformity in the conclusions. Thus, according to Blair (1935), on heating a frog nerve from zero to 30° C the rheobase increased almost twofold, i.e., the results agree with the data of L. and M. Lapicque (1907). Similar data were obtained by Zhukov and Dontsova (1949) on studying the excitability of heart muscle; intersection of the intensity-time curves were observed in their experiments as in those of Lapicque, because the threshold of short-term excitability decreased with heating, while the rheobase increased. However, Colle (1934), in experiments on the same tissues, described a lowered rheobase on heating. Decrease in rheobase and in short-term excitability threshold with rise in temperature was also
315 observed by Krigsman, von-der-Kamer, van-Lennep and Stolk (1950) on a frog nerve. At the same time, Schoepfle and Erlanger (1941) working with isolated fibers of a frog nerve, showed that with a stimulus of 0.1 milliseconds' duration, excitability actually did not depend on temperature (the fluctuations did not exceed 10%). Finally, Strohl and Djourno (1942) described an initial decrease in rheobase of several seconds) following further cooling of a frog nerve.

---

* A review of the earlier literature may be found in the paper by Gotch and MacDonald (1896).

Our aim was to investigate thoroughly this complicated problem (Nasonov and Suzdal'skaya, 1956a) using a new method instead of chronaximetry. A frog nerve-muscle preparation was used, as in the majority of studies on this problem. One of the most important sources of possible error in experiments on cooling is the drying of the nerve which invariably takes place even in the most ideal moist chambers, since on lowering the surrounding temperature the nerve will always be slightly warmer for a certain time than the air in the chamber, and will therefore lose its water. On heating the chamber, on the other hand, the nerve will always be cooler than the surrounding air, which will invariably lead to condensation of distilled water on its surface*. Drying and swelling are very potent agents affecting excitability. Thus, our experiments were all conducted in vaseline oil in which the excitability of the nerve remained the same for hours. In the moist chamber drying and swelling were completely eliminated. Further, in order to avoid drying in the nerve segment bordering the cooled one, care had to be taken to avoid changing the temperature even of a small part of the nerve. Instead, the whole preparation was cooled in vaseline oil. We started from the fact that the impulse originating under the electrode necessarily reached the muscle, causing it to contract. Consequently, the appearance of a moving impulse would always be recorded at any temperature, since in our experiments there was always a response.

The instrument used in these experiments is illustrated in Figure 178. A thin-walled glass chamber (a) 3.5 cm in diameter and 3 cm high, was sealed by a stopper through which a thermometer (c) and 2 silver electrodes (d) were inserted. The muscle (e), the nerve of which was led through the electrodes in such a manner that a piece of the spine at the opposite end of the nerve hung freely from the anode, was placed on the bottom of the chamber. The chamber was filled with vaseline oil and fixed by the thermometer to the clamp of a support supplied with a movable arm. By this means the whole chamber could be raised and lowered without displacing the nerve on the electrodes. The chamber was immersed and taken out of the vessel (b)
316 in which the required temperature was maintained within one 1° C fluctuations by the addition of warm or cold water, or by addition of ice cubes. Cooling from 20 to 5° C (or heating from 5 to 20° C) of the vaseline oil was carried out for 5-8 minutes.

Excitability was measured by the above method (Nasonov and Rozental', 1953, 1955, 1956). In certain cases the whole intensity-time curve was plotted. When it was necessary to follow a rapidly changing excitability, observations were limited to determination of values a and b.

The rheobase was determined by the usual method with condenser discharges of 90 microfarads. To determine the constant 'a' in absolute units (Nasonov and Rozental', 1955, 1956) condenser discharges of a capacity of 0.01 microfarads (0.0004 milliseconds), at which about 20-30 volts are necessary to obtain a propagating impulse in the tissue, were used in all cases.

To obtain the constant 'a' in the suggested absolute units (millivolts·millisecond) we determined the number of millivolts necessary to obtain excitation at the chosen duration of current of 0.0004 milliseconds, and the two

---

* This was especially difficult to avoid on cooling or heating. A tube was used through which the nerve passed, as is often done in such experiments.

values were multiplied. Table 60 shows the absolute values of constants 'a' and 'b', and their changes in percent of initial values after changing the temperature. For the sake of simplicity Tables 61 and 65 show only the latter, since the absolute values of the constants characterizing nerve-excitability are of no special interest. In certain experimental series a resistance of 60 k ohm was introduced into the chain and the electrodes were removed 7 to 15 millimeters in order to stabilize the energy of the stimulating current (Nasonov, 1955).

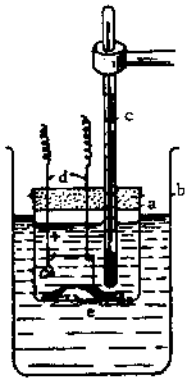


FIGURE 178. Chamber for measuring excitability of a frog nerve at different temperatures (according to Nasonov and Suzdal'skaya, 1956a) For explanation see text.

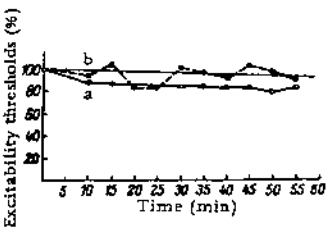
Since all the studies were performed on a nerve immersed in vaseline oil, it was first necessary under these conditions to measure the change in excitability of the nerve-muscle preparation at room temperature (20° C). The results of one such experiment are given in Figure 179. The fluctuations from the initial value in both directions do not exceed 15%. Similar fluctuations of excitability are obtained with a preparation placed in a moist chamber.

Subsequently, the effect of temperature change on nerve excitability within the range of 20-5° C and 5-20° C was studied. These experiments were carried out in April, May, June and September in the following manner. After having been isolated, the nerve-muscle preparation was kept in Ringer's solution at room temperature (20° C) for 30 minutes, and then placed in a chamber filled with vaseline oil as described above. The nerve was kept in this chamber at a temperature of 20° C for 10-15 minutes, until a constant level of thresholds 'a' and 'b' was established. After this, the entire intensity-time curve was determined. The chamber was then transferred to a vessel with water and ice where its temperature dropped to 5° C within 10-15 minutes. The temperature in the external vessel was maintained at 5°C, with a precision of 1° C. The intensity-time curve was again determined, and the dynamics of the change in values of 'a' and 'b' was followed until constant levels were established. Subsequently, the temperature was increased in the reverse order, from 5-20° C, using the same method. The values of 'a' and 'b' were again determined.

In Figure 180, 2 voltage-time curves from one experiment are illustrated in logarithmic coordinates. This shows that on cooling, the value of 'b' decreased considerably, i.e., the excitability of the nerve in this region increased. At the same time, the constant 'a' increased. This means that for shorter stimuli, excitability decreased. As a result, the two curves intersected in a region corresponding to 0.1 millisecond. These facts agree with a number of data in the literature (Waller, 1899, Lapicque, 1907, Blair, 1935, Zhukov and Dontsova, 1949), but at first sight they seem highly paradoxical and require special explanation, since it would be natural to expect that on cooling, excitability would decrease for impulses of any duration.

Figure 181 shows the same phenomena after processing of 'a' and 'b'. It will be seen that cooling the nerve caused an opposite reaction in these thresholds. The former increased twofold, while the latter decreased by 15%. In the reverse procedure (from 5 to 20° C) the excitability thresholds of this preparation almost returned to initial conditions. This peculiar

temperature reaction of the nerve was confirmed, with certain variations, in a great number of experiments (more than 30) during the spring and summer months.



318 FIGURE 179. Change in thresholds of short-term (a) and prolonged (b) excitability of frog nerve when the preparation is kept 1 hour in vaseline oil at a temperature of 20° C (according to Nasonov and Suzdal'skaya, 1956a). The initial level of excitability was taken as 100%.

The data in Table 60 show that on cooling a nerve in April frogs, the short-term threshold of excitability markedly increased. This could have been expected since it is natural to assume that decrease in temperature will decrease excitability. The protracted threshold was slightly lowered, i.e., excitability of the nerve with respect to this index increased. On heating, reverse relationships were observed. The mean square errors were less than one-third of the mean values (except for the last column), which proved their statistical significance.

Table 61 gives similar data for May, June and September. From these figures it can be seen that the rule that the thresholds of prolonged and short excitations change in opposite directions after temperature changes, while intensity-time curves intersect each other, is even more obvious. In all cases the mean values are statistically well founded.

In addition to the above experiments, the excitability of the nerve after temperature change was studied in May according to the same schedule: i.e., changes from 20 to 30° C and vice versa (Table 62).

Table 62 shows that the above rule is also applicable to a nerve heated to 30° C. Here, too, excitability due to short electric stimuli (a) increased after heating, while (b) decreased after prolonged stimuli, a result which again disagrees with the usual observations on the effect of temperature. This was not seen on cooling from 30 to 20° C (there is only a drop of 0.5% in the threshold level). This can, however, be logically explained since keeping the nerve at 30° C for 5-10 minutes causes a certain damage.

In the literature, various authors commenting on the effect of temperature on excitability point out that changes in temperature should markedly affect electroconductance of the nerve. Because of this, when 319 excitability is evaluated by ordinary methods using a threshold voltage, there is a considerable margin of error. This is because at a constant voltage the threshold intensity of the current changes proportionally to the resistance of the nerve. Since the intensity of the current is considered responsible for the appearance of excitation, this intensity is stabilized and made independent of the resistance of the nerve by introducing a high resistance into the chain. But we succeeded in showing that this was not enough. It was not the intensity, but the energy of stimulating current, which was responsible for the appearance of excitation in the nerve. It was necessary to ensure a high resistance of the chain (50-100 k ohm), and a sufficiently large distance between the stimulating electrodes (approximately 10-15 mm) (Nasonov, 1955).

Experiments carried out in September, during which these two conditions were observed, showed that the results did not fundamentally differ

from those previously obtained (compare Tables 63 and 61). Therefore, the conclusions regarding the effect of temperature on excitability cannot be designated as artifacts caused by an altered resistance in the nerve.

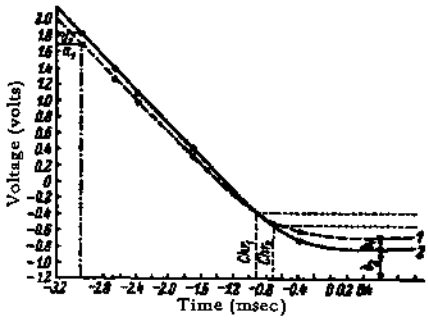


FIGURE 180. Logarithmic voltage-time curves of a nerve of autumn frog (September), on change in temperature (according to Nasonov and Suzdal'skaya, 1956a)

1—at 20° C; 2—at 5° C; a—threshold of short-term excitability; b—rheobase; Chr-chronaxy. The curves intersect in the region of 0.1 milliseconds (log 0.1 = 1.0).

On this basis it may be considered an established fact that following changes in temperature within the range of 30-20° C and 20-5° C, the excitability of nerves of spring, summer and autumn frogs obeys the following rules:

a) decrease in temperature causes an increase in the short-term threshold of excitability (constant 'a'), and a decrease in the prolonged threshold (constant 'b');

b) increase in temperature leads to opposite results;

c) the intensity-time curves intersect. This means that within the limits of certain time intervals (about 0.1 milliseconds) the excitability threshold does not depend on temperature. The region of intersection between the curves may be seen to coincide approximately with the time of action-current of the moving excitation peak (duration of ascending part of the peak). Consequently, excitability corresponding to the optimal time of excitation is protected from the effect of temperature. In reviewing the literature, the study of Schoepfle and Erlanger (1941) showed that on stimulation of a frog nerve by current impulses close to the duration of the propagation peak (0.1 millisecond), the excitability of the nerve hardly changed upon cooling. (Note: on cooling by 12° C the threshold rose by approximately 10%).

In our opinion, coldblooded animals in which the body temperature is not constant possess a certain mechanism to maintain constant excitability of the nerves. If this were not so, then a summer frog, moving from a shallow water basin heated by the sun into that part where a cold stream
322 enters it, should be immediately immobilized, due to loss of conductivity. This is actually not the case.

If the above suggestion is correct, we might expect that in frogs wintering under the ice at a constant temperature close to zero, intersection of the excitability curves of the nerve cannot be detected, which, in our opinion, is an expression of adaptation to rapid changes of the surrounding temperature.

Observations made in February and March confirmed this assumption.

As can be seen from Table 64, change in nerve excitability of February frogs upon cooling from 20 to 5° C is characterized by decreased excitability at all points of the intensity-time curve (increase in 'a' and 'b'). There is no intersection of the curves and consequently, there is no point which does not depend on temperature.

Table 60

Excitability constants of nerve in frogs caught in April, after changes in temperature (according to Nasonov and Suzdal'skaya, 1956a)

Initial value of a (mvolts·msec)	From 20 to 5° C			From 5 to 20° C (same preparation)			
	Change in a (% of initial value)	Initial value of b (volts)	Change in b (% of initial value)	Initial value of a (mvolts·msec)	Change in a (% of initial value)	Initial value of b (volts)	Change in b (% of initial value)
12.8	+ 81	0.12	−17	23.2	−59	0.10	+17
8.2	+ 36	0.12	−17	15.2	−42	0.10	0
12.8	+100	0.14	−14	24.8	−58	0.16	0
7.2	+111	0.10	0	15.2	−58	0.10	0
16.0	+ 75	0.20	−10	28.0	−50	0.18	0
16.8	+ 90	0.94	− 9	32.0	—	0.22	—
13.6	+100	0.12	−17	27.2	—	0.10	—
13.6	+117	0.16	−12	29.6	—	0.14	—
9.6	+108	0.16	−12	20.0	−52	0.14	0
10.4	+115	0.14	−15	22.4	−57	0.12	+14
7.2	+ 88	0.12	−34	13.6	−30	0.08	+20
12.0	+126	0.18	−10	27.2	—	0.16	—
8.8	+109	0.12	0	18.4	−57	0.12	0
12.0	+ 87	0.10	0	24.0	−58	0.08	−20
Arithmetic means							
11.5	+96 ± 8	0.19	−11.9 ± ± 0.7	22.9	−52.1 ± ± 3	0.13	+3.1 ± ± 3.6

Analysis of Table 65 reveals the seasonal nature of temperature changes of excitability constants. In February and March, when the frogs are taken from under the ice, the temperature changes of 'a' and 'b' are in the same direction and consequently the curves do not intersect. However, the change in 'a' is considerably greater than that of 'b'. From April to September, when the frogs move freely, opposite changes are observed in the constants. Consequently, intersection of the curves takes place and there is a mechanism which stabilizes excitability following temperature changes. This is less strongly expressed in April, but most marked from May to September, when the frogs experience most rapid changes in the surrounding temperature*.

What explanation is there for the fact that excitability determined by prolonged impulses (b) increases upon cooling, but decreases when acted

---

* It is highly probable that the seasonal nature of the temperature reaction of excitability is one of the causes for contradictions between the different investigators who studied the problem under review.

on by short impulses of current. To explain this, we postulated that the metabolism of nerve fibers initiated by an electrical stimulus plays the
323 role of a regulator of nerve excitability. It also restores the initial condition of the fiber after passage of the excitation wave.

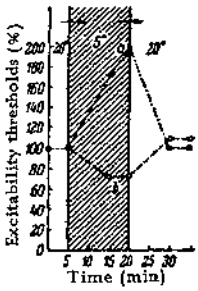


FIGURE 181. Thresholds of short-term (a) and prolonged (b) excitability of a nerve of spring (May) frog, on changing the temperature from 20 to 5° C and back (according to Nasonov and Suzdal'skaya, 1956a). The initial excitability level is taken as 100%.

In order to achieve this chain of metabolic reactions, a certain time is required. The shorter the time of action of the excitant, the less possibility is there for these reactions to increase excitability during lowering of temperature, and as a result, excitability decreases (increase in 'a'). If the time of action of the stimulus is too long, the compensatory metabolic activity leads to an excessive increase in excitability (decrease in 'b'). At certain intermediate time intervals, close to the physiological norm, metabolic activity maintains nerve excitability at a more or less constant level, and guards against sudden increase in nerve excitability.

To check these postulates, we tried to eliminate one metabolic link, i.e., carbohydrate breakdown, by treating the nerve with monoiodoacetic acid. The experiments were carried out in April.

An isolated nerve-muscle preparation was kept for 30 minutes in Ringer's solution. A small segment of the nerve, which was subsequently brought in contact with the electrode, was immersed for 30 minutes in 2% neutralized solution of monoiodoacetate in Ringer's solution. Subsequently the preparation was transferred to a chamber in which its excitability was measured by the above method at 20° C, at 5° C and then again at 20° C. Figure 182 shows the results of such an experiment. Here, as in other cases described above, on cooling the preparation from 20 to 5° C, constant 'a' increased, while constant 'b' decreased (Figure 182, B). If the preparation again was heated to 20° C, it returned to its initial condition. Figure 182, A shows a change in excitability of a nerve-muscle preparation, which was a duplicate of the previous preparation and which was also treated with 2% monoiodoacetate. Comparison with the previous preparation gives striking results. Constant 'a' increased markedly on cooling (in Figure 182, A-B, 10-fold). However, this increase was not a simple result of nonspecific poisoning by monoiodoacetate, since on heating from 5 to 20° C, constant 'a' returned to its initial value. The change in constant 'b' on cooling differed qualitatively from the control. It did not decrease as in the former case, but increased 2-3-fold, returning to its initial value on heating.

The results were easily explained in terms of the above hypothesis. As a result of elimination of carbohydrate metabolism, both thresholds of excitability increased on cooling, this being especially noticeable in the case of 'a'. There was no intersection between the curves, and consequently
324 decrease in excitability took place along the front of the intensity-time curve. There was no region of time intervals during which excitability did not depend on temperature. Evidently, metabolic processes stabilize the excitability of the nerve with change in temperature.

Table 61

Excitability constants of nerve of frog caught in May, June and September, with changes in temperature (according to Nasonov and Suzdal'skaya, 1956a)

From 20 to 5° C		From 5 to 20° C (same preparation)	
a	b	a	b
% of initial value			
+114	−23	−48	+57
+ 75	−28	−50	+62
+ 91	−15	−51	+16
+ 30	−23	−40	+25
+ 44	−12	−63	+14
+ 17	−20	−49	+ 7
+ 64	−10	−40	+40
+ 66	−29	−43	+33
+ 73	−25	—	—
Arithmetic means			
+63.7 ± 10.0	−20.6±2.3	−48 ± 2.6	+31.8 ± 7.1

Table 62

Excitability constants of nerve of frog caught in May, with changes in temperature (according to Nasonov and Suzdal'skaya, 1956a)

From 20 to 5° C		From 5 to 20° C (same preparation)	
a	b	a	b
% of initial value			
−12	+43	+ 7	0
−25	+37	—	—
−23	+29	+28	−10
−10	+25	—	—
−34	0	—	—
−17	+12	+30	+11
−28	+10	+41	+10
−15	+25	+25	0
−36	+12	+25	−14
Arithmetic means			
−22.2 ± 3.08	+21.4 ± 4.6	+26.5 ± 4.9	−0.5 ± 4.1

Table 63

Excitability constants of nerve of frog caught in September, with changes of temperature. An additional resistance of 6,000 ohm was introduced into the chain, the distance between the electrodes being 15 mm (according to Nasonov and Suzdal'skaya, 1956a)

From 20 to 5° C		From 5 to 20° C (same preparation)	
a	b	a	b
% of initial value			
+59.0	−20.0	−33.0	+25.0
+41.7	−33.4	−23.5	+20.0
+35.7	−18.4	−26.3	+20.0
+60.0	−14.3	−33.4	+33.3
+44.0	−25.0	−34.0	+50.0
+32.0	−30.0	−30.0	+57.0
+44.0	−40.0	−25.0	+38.0
Arithmetic means			
+45.2 ± 4.06	−25.9 ± 3.4	−29.3 ± 0.56	+34.7 ± 5.49

Table 64

Excitability constants of nerve of frog caught in February, with changes in temperature (according to Nasonov and Suzdal'skaya, 1956a)

From 20 to 5° C		From 5 to 20° C (same preparation)	
a	b	a	b
% of initial value			
+ 12	0	−43	−34
+ 40	0	−47	0
+ 91	+50	—	—
+ 50	+50	−50	−34
+ 57	0	−36	−43
+100	0	−34	−15
+ 73	+40	—	—
+120	+33	−46	−67
+ 47	+20	−40	−20
+100	+25	−45	−25
Arithmetic means			
+69.1 ± 10.6	+21.8 ± 6.6	−42.6 ± 0.6	−29.7 ± 7.1

Table 65

Excitability constants of frog nerve, with changes in temperature (according to Nasonov and Suzdal'skaya, 1956a)

Month	From 20 to 5° C		From 5 to 20° C (same preparation)	
	a	b	a	b
	% of initial value			
February	+69.1	+21.8	−42.6	−29.7
March	+82.0	+10.0	−15.0	−15.0
April	+96.0	−10.0	−51.6	+ 3.7
May, June, September	+62.7	−20.0	−48.0	+31.8

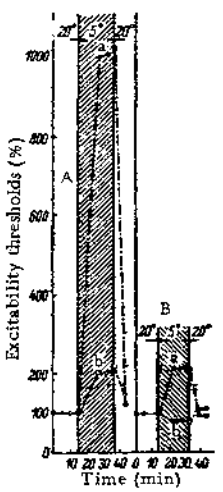


FIGURE 182. Thresholds of short-term (a) and prolonged (b) excitability of frog nerves with change in temperature from 20 to 5° C and back (according to Nasonov and Suzdal'skaya, 1956 a)

A–action of 2% monoiodoacetate; B–control second nerve not treated thus). The initial level of excitability was taken as 100%.

The data obtained with the frog nerve were checked with the nerve of another cold-blooded animal–the marsh toad (Emys orbicularis (Nasonov and Suzdal'skaya, 1956 c). The toad was immobilized by destroying the spinal cord with a needle, after which the lower armor fold and all the viscera were removed. Subsequently the sciatic nerve was exposed for 2-3 cm, the proximal end was cut and its excitability determined in a stream of vaseline oil heated to a certain temperature, as was done with rat and pigeon nerves (Figure 185). The investigation of excitability in the temperature range from 20 to 10° C invariably gave identical results (Figure 183). As in the summer frog, excitability of the tortoise nerve after prolonged stimulation increased on cooling. During short intervals of stimulation it decreased, and therefore the intermediate, most nearly physiological region of the intensity-time curve was noted to be independent of temperature.

Figure 184 illustrates the dynamics of change of 'a' and 'b' on decrease and increase in temperature, and indicates that both constants change in opposite directions.

Thus, the intersection of the intensity-time curves of nerves of cold-blooded animals may be considered as an expression of a special substitution of thermoregulation.

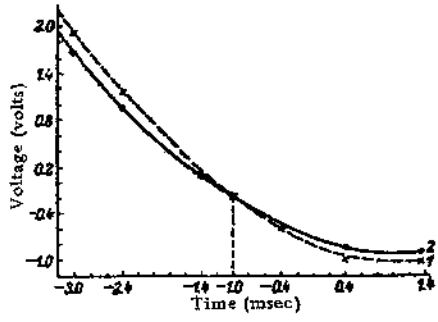


FIGURE 183. Logarithmic curves of voltage-time of tortoise nerve; on changes in temperature (according to Nasonov and Suzdal'skaya, 1956c)

1-at 10°C; 2-at 20°C. The point of intersection is designated by a vertical line.

Experiments with Nerves of Warm-Blooded Animals (Rats, Pigeons)

In the previous chapter it was shown that on cooling the nerves of a summer frog and toad, their prolonged threshold of excitability decreased, while the short-term threshold rose. With heating, opposite changes took place. These facts are in agreement with numerous data from the literature. It has also been shown that on poisoning of a frog nerve with monoiodoacetate, both thresholds increased on cooling. They also increased on cooling of nerves of winter frogs.

A suggestion was put forward that these facts are an expression of a peculiar adaptation of the nerves of cold-blooded animals, maintaining constant excitability during changes in the environmental temperature. In warm-blooded animals possessing thermoregulation, there is no need for such a stabilizing mechanism. It was therefore suggested that both excitability constants 'a' and 'b' will increase on cooling and decrease on heating of the nerve.

This was checked by us experimentally (Nasonov and Suzdal'skaya, 1956b, 1956c, 1958) using the sciatic nerve of a white rat. The animal was narcotized and fixed in prone position on a mount. The sciatic nerve was exposed for 2-3 cm and cut. A thread which was passed through special orifices (c and d) in the glass chamber by the use of a needle (Figure 185) was tied to the end of the central end of the nerve. Following the thread, the end of the nerve (i) was introduced into the chamber and was placed in-
326 side it on electrodes soldered to its upper wall (e and f). The distance between the electrodes was 1 cm. The orifices of the chamber (c and d) were subsequently sealed by clay made in a base of Ringer's solution. Subsequently a stream of vaseline oil heated to the required temperature was passed through the chamber via tubes (a and b).

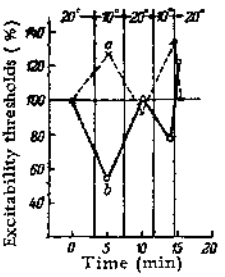


FIGURE 184.
Thresholds of short-term (a) and prolonged (b) excitability of tortoise nerve; on changing temperature from 20 to 10° C and back (according to Nasonov and Suzdal'skaya, 1956c) Initial excitability is considered as 100%.

The excitation induced in the nerve by means of electric stimuli was recorded by the movement of the hind toe, in which normal blood circulation was maintained during the experiment. The stimulating current was supplied by the apparatus described above, whereby a resistance of 60 k ohm was introduced serially into the chain. During each experiment, the rats were kept under narcosis, and remained alive while normal blood circulation was maintained. The rectal temperature of the animals was about 34° C.

First it was determined to what extent the excitability constants of the nerve changed under experimental conditions at a constant temperature. For this purpose, the end of the nerve was threaded through the orifices of the chamber, a stream of vaseline oil heated to 21° C was introduced, and the whole curve of voltage-time was determined every 15-30 minutes.

Figure 186 shows the dynamics of these values for the duration of 4 hours. During the initial 30 minutes the excitability of the nerve hardly changed. After 40 minutes both excitability thresholds began to increase, and at about 4 hours threshold 'b' increased 8-fold, while constant 'a' increased 4-fold. Consequently, both excitability thresholds of the nerve and the threshold time of excitability necessary for the appearance of the reaction, were found to increase. At the same time chronaxy shortened and at about 4 hours decreased more than 2-fold. The latter fact again indicates that chronaxy is not a measure of the threshold time of excitation, as is usually thought.

It has been shown earlier (Figure 172) that with time, there is an increase in threshold intensity and the time of excitation necessary for appearance of a reaction at all the points of the voltage-time curves, with chronaxy shortening in the process. This may be explained by the fact that increase in rheobase 'b' (right-hand horizontal wing of the curve) occurred more rapidly in this case than the increase in constant 'a' (left-hand sloping wing of the curve). And since chronaxy equals the ratio of 'a' to 'b', it became shorter, notwithstanding the fact that the threshold time of appearance of a reaction noticeably increased.

In the above preliminary experiments, the important fact is that for the first 30-40 minutes, excitability of the surviving nerve remained constant, while the duration of the experiments described below did not exceed 20-30 minutes.

Turning to the experiments, we first of all investigated the effect of temperature change from 21 to 30° C and back on excitability. In the above-described chamber, the rate of this change may be 2-3 minutes. We changed the temperature within 5 minutes*.

In some experiments the whole voltage-time curve was determined; however, in the majority of cases we limited ourselves to determination of

---

* As a result of specially designed experiments, we have seen that the speed of temperature change does not appreciably affect the result.

the constants 'a' and 'b', for which purpose it is necessary to perform only two measurements. At the beginning of each experiment two measurements were taken at 21° C, at 5-minute intervals. Subsequently, when we were assured that the value of the two constants did not change, vaseline oil, heated to 30° C, was introduced and the measurement was repeated at this
327 temperature. Subsequently, we again lowered the temperature to 21° C and again measured the excitability constants.

The results of these experiments are shown in Table 66.

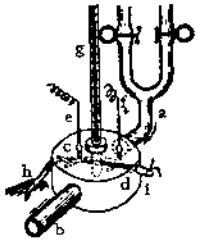


FIGURE 185. Glass chamber for study of effect of temperature on nerve excitability (according to Nasonov and Suzdal'skaya, 1956b); a and b—tubes through which a stream of vaseline oil at the required temperature was passed; c and d—orifices through which the nerve was threaded; e
328 and f—electrodes; g—thermometer.

Table 66 shows that on increase in temperature from 21 to 30° C, the value of both excitability constants decreased ('a' by 41%, 'b' by 30%), while on cooling to the same temperature it increased, returning to the initial value. Consequently, in the rat nerve, on changing temperature from 21 to 30° C, no intersection of the curves was observed by us or by other authors working with nerves of summer frogs. At all points of the intensity-time curve there was an increase in excitability on heating and a decrease on cooling (Figure 187).

Exactly the same results were obtained on changing the temperature from 25 to 35° C and back.

It must be mentioned that alterations of rat nerve excitability due to temperature changes within the range from 25 to 35° C is very easily reversible. Obviously, cooling the nerve to 20° C causes no serious damage.

We also studied the change of excitability constants within the range 35-40° C (Nasonov and Suzdal'skaya, 1958). On heating a rat nerve from 35 to 40° C, excitability decreased according to both indexes 'a' (37%) and 'b' (17%). The question arose: is this decrease due to the fact that at 40° C metabolic processes are much more intense and there is not enough oxygen in the vaseline oil? Indeed, on aeration of the vaseline oil, different results were obtained. On increase of temperature from 35 to 40° C, constant 'a' decreased (−15 ± 2.9%), and constant 'b' increased (+36 ± 5.8%). On cooling from 40 to 35° C, constant 'a' increased (+13 ± 2.9%), while constant 'b' decreased (−22 ± 2.4%).

Thus, an intersection of the voltage-time curves of the nerve of warm-blooded animals was observed after temperature changes within a narrow region of 35-40° C.

Later we investigated the change in excitability of a rat nerve when temperature varied between 20-5° C. The results are shown in Table 67.

329 It follows from Table 67 that cooling of a rat nerve from 20 to 5° C led to a marked increase in excitability constants ('a' 13-fold, 'b' 11-fold). Heating of the same nerve from 5 to 20° C caused a drop in both constants. However, complete reversibility of the process did not occur here as in the temperature range 20-30° C. Constant 'a' returned only to a level of 140%

---

* In other temperature ranges aeration of the vaseline oil did not affect the results obtained (Nasonov and Suzdal'skaya, 1958).

of the initial value, and constant 'b' only to 187%. Obviously, even short cooling of the rat nerve to 5° C left a damage which was not easily reversible. All these phenomena are well seen in Figure 188.

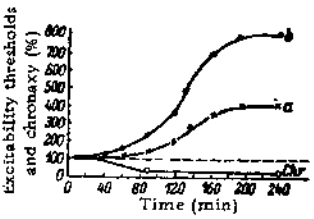


FIGURE 186. Change in thresholds of short-term (a) and prolonged (b) excitability and chronaxy (Chr) of peripheral segment of rat nerve (according to Nasonov and Suzdal'skaya, 1956b). The initial level of excitability and chronaxy was taken as 100%.

Nevertheless, in principle the same changes occur in the temperature range of 20-5° C as in the 30-20° C range, namely, on cooling, both excitability constants increase while on heating they decrease. Consequently, here, too, there is no intersection of the intensity-time curves.

A pigeon was used to determine the effect of temperature on excitability of the sciatic nerve by exactly the same method as in the case of the rat.

Excitability of the pigeon nerve increased at all points of the intensity-time curve on increasing the temperature from 20 to 30° C and from 30 to 40° C, while on cooling it decreased. At 10° C the nerve of the pigeon lost its conductance capacity. Figure 189 B shows the dynamics of change of constants 'a' and 'b' on repeated transition from 20 to 30° C and back. Both constants changed in an exactly similar manner and the reversibility of these changes was exceptionally complete.

Thus, experiments on the effect of temperature on nerve excitability show that on cooling of a summer frog nerve, its excitability constant 'a' increased, while constant 'b' decreased. On heating, opposite changes of the constants took place. As a result, the voltage-time curves of the frog nerve intersect with each other after change in temperature. The point of
330 intersection determines the duration of the stimulus with which the excitability does not change as a result of temperature changes.

We postulated that the intersection of voltage-time curves is an expression of a specific adaptation, due to which excitability of the nerve of cold-blooded animals measured in physiological time intervals changes only slightly with changes in temperature of the surrounding medium. We consider this to be a substitute for thermoregulation. In the rat, cooling of the nerve within the temperature ranges 30-20° C and 20-5° C causes an increase in both constants, while heating of the nerve leads to their decrease. A similar picture was observed in the pigeon in the ranges of 30-20° C and
331 40-30° C. In other words, on cooling, excitability of the nerve decreases along the whole intensity-time curve and increases upon heating.

For rat and pigeon nerves, excitability depends on temperature for the entire threshold excitation in the above-given temperature limits. Intersection of the voltage-time curves, and therefore regulation of excitability as applied to temperature, are possible in the case of warm-blooded animal nerves only within a very narrow temperature range (for rat nerves 35-40° C).

Table 66

Excitability constants of rat nerve during changes in temperature (according to Nasonov and Suzdal'skaya, 1956b)

21° C		30° C		21° C	
a (in mvolts msec)	b (in volts)	a (in mvolts·msec)	b (in volts)	a (in mvolts·msec)	b (in volts)
26	0.08	6	0.02	30	0.12
28	0.08	18	0.06	32	0.14
22	0.04	18	0.03	24	0.08
26	0.10	12	0.08	24	0.08
20	0.08	10	0.04	16	0.06
34	0.18	26	0.16	30	0.22
20	0.06	13	0.04	19	0.06
42	0.20	26	0.14	38	0.20
20	0.10	12	0.06	17	0.08
Arithmetic means					
26.4 ± 2.51	0.10 ± 0.02	15.6±2.12	0.07±0.01	25.5± 2.49	0.11±0.05
% of initial value		59	70	96.6	110

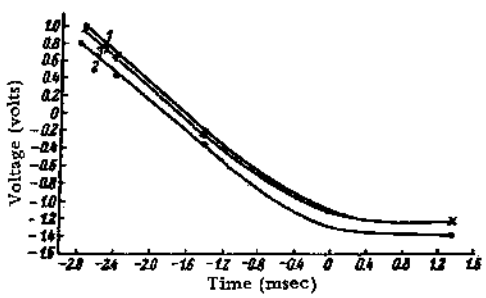


FIGURE 187. Logarithmic voltage-time curves of a rat nerve with changes in temperature (according to Nasonov and Suzdal'skaya, 1956b).

1—at 21° C; 2—at 30° C; 3—after returning to the initial temperature, 21° C.

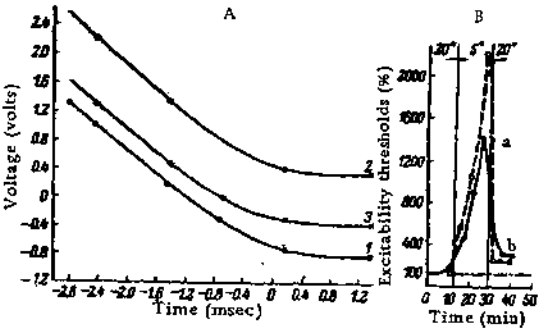


FIGURE 188. Excitability of rat nerve with change in temperature from 20 to 5° C and back (according to Nasonov and Suzdal'skaya, 1956b)

A—logarithmic voltage-time curves: 1—at 20° C; 2—on cooling to 5° C; 3—after returning to the initial temperature, 20° C. B—changes in thresholds of short-term (a) and prolonged (b) excitability. The initial level of excitability was taken as 100%.

Table 67

Excitability constants of rat nerve after change in temperature (according to Nasonov and Suzdal'skaya, 1956b)

20° C		5° C		20° C	
a (in mvolts msec)	b (in volts)	a (in mvolts·msec)	b (in volts)	a (in mvolts msec)	b (in volts)
56	0.16	336	1.04	64	0.26
64	0.26	336	1.40	84	0.48
27	0.18	208	0.84	52	0.26
40	0.20	—	—	—	—
32	0.10	1072	3.50	53	0.24
38	0.14	400	2.04	76	0.40
30	0.10	1152	3.20	15	0.24
16	0.06	204	0.40	32	0.08
28	0.12	240	0.80	40	0.28
		Arithmetic means			
36.7±1.13	0.15±0.064	493±119.8	1.65±0.4	52.0±5.56	0.28±0.01
% of initial value		1344	1100	141	187

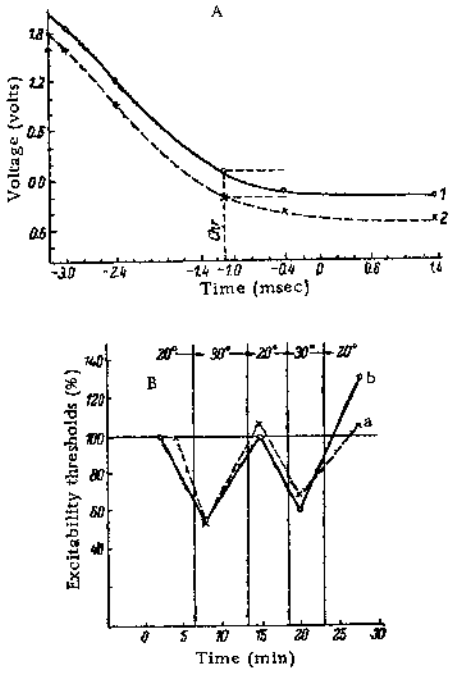


FIGURE 189. Excitability of pigeon nerve with change in temperature from 20 to 30° C and back (according to Nasonov and Suzdal'skaya, 1956c)

A—logarithmic voltage-time curves: 1—at 20° C; 2—at 30° C. B—changes in thresholds of short-term (a) and prolonged (b) excitability. The initial level of excitability was taken as 100%.

Experiments with Skeletal Muscles of Frog and Rat

The effect of temperature on excitability of skeletal muscles in cold-blooded (frog) and warm-blooded animals (rat) was studied by Suzdal'skaya (1957a, 1957b). A sartorius muscle of the frog was studied, and its excitability in vaseline oil was investigated in the above-described chamber (p. 307). After investigation of the frog muscle at 30° C an irreversible drop in excitability was often observed, and therefore the effect of temperature on excitability thresholds was studied within the ranges of 25-15° C and 21-7° C. The experiments were conducted in the same manner as in the study of the nerve. The muscles were placed on electrodes (inter-electrode distance—18 mm) in such a manner that the cathode was in contact with the nerveless segment of the muscle. A resistance of 20 k ohm

was introduced serially into the chain. The results of the experiment are shown in Tables 68 and 69, in both of which the arithmetic means from 10 experiments are set out.

Table 68 shows that on increasing temperature from 15 to 25° C, the threshold of short-term excitability falls by 20 %, while the threshold of protracted excitability rises by 46 %. The same muscles cooled again show a rise in 'a' by 11 % (incomplete reversibility) and a fall in 'b' by 32%. It follows that the voltage-time curves of frog muscles intersect exactly in the same manner obtained with nerves.

The same results are obtained within the temperature range of 21-7° C (Table 69). Here, on cooling, a 65% rise of 'a' and 39% fall in 'b' occur. On heating the same muscles, 'a' falls by 32.5 % and 'b' rises by 64.3 %. Consequently, for frog muscles, as for frog nerves, the same range (of duration of stimulating current) exists, at which excitability does not depend on temperature. This range lies within physiological limits. Consequently, the excitability of the muscles of a cold-blooded animal
332 adapts to changes in temperature, replacing the thermoregulation of warm-blooded animals.

The effect of temperature on the excitability of skeletal muscles of a white rat was investigated on m. soleus, using the same method as that employed in the case of frog muscles. The results of the experiment for the range 20-10° C and back are given in Table 70.

There is no intersection between the excitability curves of rat muscles, just as there is no such intersection in the case of rat nerve within this temperature range. On cooling, both constants markedly increase in value, 'a' considerably more so than 'b' (Table 70). Consequently, no adaptive mechanisms exist for stabilizing excitability during a temperature rise.

However, within the range of 20-30° C a different picture is sometimes observed (Table 71). Here the excitability curves clearly intersect and therefore there may exist a certain stabilization of excitability of muscles on decrease in temperatures not below 20° C. Below this temperature the rat muscles lose the capacity to regulate their excitability.
333 Probably on the appearance of homeothermia during the process of evolution, nerves lost the capacity to regulate excitability following changes in temperature to a much greater extent than muscles.

Table 68

Change in excitability constants ('a' and 'b') of sartorius muscles of a frog within the temperature range 25-15° C. Arithmetic means from 10 experiments (according to Suzdal'skaya, 1957a)

a (in mvolts-msec)			b (in volts)			a (in mvolts-msec)			b (in volts)		
15°	25°	% of initial value	15°	25°	% of initial value	25°	15°	% of initial value	25°	15°	% of initial value
152.4	121.9	-20.0± ±4.2	0.90	1.31	+46.0± ±3.9	121.9	136.0	+11.4± ±2.0	1.2	0.82	-32.0± ±2.0

Table 69

Change in excitability constants ('a' and 'b') of sartorius frog muscles within the temperature range 21-7° C. Arithmetic means from 10 experiments (according to Suzdal'skaya, 1957a)

a (in mvolts·msec)			b (in volts)			a (in mvolts msec)			b (in volts)		
21°	7°	% of initial value	21°	7°	% of initial value	7°	21°	% of initial value	7°	21°	% of initial value
153	252	+65 ± ±11	0.92	0.56	-39.0 ± ±2.8	252	170	-32.5 ± ±3.9	0.56	0.92	+64.3± ±8.7

Table 70

Change in excitability constants ('a' and 'b') of m.soleus of a white rat within the temperature range 20-10° C. Arithmetic means from 10 experiments (according to Suzdal'skaya, 1957b)

a (in mvolts·msec)			b (in volts)			a (in mvolts·msec)			b (in volts)		
20°	10°	% of initial value	20°	10°	% of initial value	10°	20°	% of initial value	10°	20°	% of initial value
407	2974	+630± ±120.8	0.88	1.91	+117± ±28	2974	624	-79± ±3.8	1.91	1.03	-46± ±4.6

Table 71

Change in excitability constants ('a' and 'b') of m. soleus of a white rat within the temperature range 20-30° C. Arithmetic means from 10 experiments (according to Suzdal'skaya, 1957b)

a (in mvolts·msec)			b (in volts)			a (in mvolts·msec)			b (in volts)		
20°	30°	% of initial value	20°	30°	% of initial value	30°	20°	% of initial value	30°	20°	% of initial value
440	354	-20± ±4.9	0.92	1.40	+52± ±8.8	472	733	+55± ±4.6	1.37	1.42	-10±3

Chapter 6. Conclusions

As far as we know, no one has measured excitability of tissues by means of the three constants, 'a', 'b' and 'n'. Therefore the study of dynamics of these constants following the effect of various agents has an important topical significance.

We have studied in greater detail the change in excitability constants of a nerve separated from CNS connections, and the effect of temperature on excitability constants of nerves and muscles. In addition, we performed a series of exploratory observations on the effect of such an agent as ethyl alcohol vapor; Ringer's solution with a 2-fold and a half concentration of NaCl; maintenance in a moist chamber for 24 hours, etc., on the excitability constants of a nerve-muscle preparation of frog.

On the basis of these hitherto incomplete data the following preliminary generalizations may be made:

1. The values of constants 'a' and 'b' change considerably following the action of each of the above-mentioned agents.

2. Changes in these two excitability thresholds occur to a considerable extent independently of each other*. Most often these constants change in the same direction. However, only very rarely are these changes proportional to each other. Sometimes one of the constants changes, while the other remains unchanged. Finally, there are cases where one increases while the other decreases. In such a case the intensity-time curves intersect with each other before the action of the corresponding agent, and after it. Such changes in excitability are observed after alterations in temperature.

3. Constant n (increment factor) was shown to be the most stable. None of the agents caused any significant change in the slope of the left-hand straight segment of the logarithmic curve of intensity-time. The rectilinear-inclined part of the graph always showed a parallel shift to itself on decrease or increase in threshold of the short-lasting excitability 'a'. The significance of this relative stability of constant 'n' is so far incompletely understood.

4. Chronaxy is not a time constant, as postulated by the majority of contemporary physiologists who studied excitability of conducting tissues. This concept of chronaxy may lead to very gross errors of interpretation of experimental results. An especially illustrative example of this is the well-known case of increased chronaxy and decreased rheobase on separation of the nerve from its CNS connections, as elaborated by us in detail. It is quite obvious that in this case, increase in chronaxy is accompanied by a decrease in the time necessary for the appearance of excitation.

In reality, chronaxy determines the range of time intervals within which the time of action of the stimulus starts to show a considerable effect on the value of the threshold of excitation.

---

* It is self-evident that in a system like the nerve cell with its dendrites, all the processes are linked to each other. In speaking of the non-dependence of constants 'a' and 'b', we only imply that their existing interconnections are unknown.

Part VI

334 CONDITIONS FOR THE APPEARANCE OF SPREADING EXCITATION IN A CONDUCTING FIBER

Chapter 1. Importance of the Amount of Electricity and Energy of Stimulating Current During the Appearance of Excitation

Theory of the Physiological Action of Electric Current

The majority of physiologists have long considered that on stimulation with electric current, the decisive factor for the appearance of excitation in a nerve conductor is the intensity of the current or the amount of electricity. In ordinary excitometric systems, the excitability thresholds are measured not in amperes but in volts. It is, however, considered that this is done merely because at very short time intervals the intensity of current cannot be measured directly by an ammeter, and since under conditions of constant resistance in the circuit, the voltage is proportional to the intensity of the current, when measuring the threshold voltage at the same time we measure the threshold intensity.

In order for the current intensity to be actually proportional to voltage, it is necessary to eliminate somehow the effect of fluctuating resistance which may arise during the experiment in the investigated tissue itself. For this purpose, higher resistance was introduced into the circuit, in comparison with which the fluctuating resistance of the tissue was so small that it could be ignored*. However, a high resistance in the circuit stabilizes only the current intensity, but not the voltage on the electrodes, the changes of which were considered not to affect the value of the threshold**.

Thus, an opinion formed according to which the intensity of the current, and not its voltage on the electrodes, determines the value of the electric threshold of excitation***.

See, for example, Chir'ev (1877), Gotch and McDonald (1896) and others. Lapicque's shunt is based on the same principle and so are numerous studies on determination of excitability performed by the use of the chronaximetric method.

** Later, Rushton (1934) used an extremely sensitive and very slightly inert galvanometer for determination of excitability, allowing direct measurements of intensity of very short current pulses (up to 0.8 millisecond). The author measured the threshold value in microamperes, not taking into account the fact that during the experiment there were also changes in the voltage at the electrodes, which Rushton obviously considered possible to disregard.

*** It should be pointed out that Stämpfli (1952) recently brought evidence indicating the decisive role of voltage on excitation by electric current.

335 Since the beginning of the 20th century this problem became more complicated, after it had become obvious that at sufficiently short time intervals not only the intensity of the current but also the duration of its passage through the fiber is of importance. On the basis of the empirical formula of Hoorweg-Weiss, it might have been assumed that in the region of short current impulses, the decisive factor for appearance of excitation was the product of current intensity and time ($i \cdot t = a$ = constant), i.e., the threshold amount of electricity (Q).

On this basis attempts were made to explain theoretically the actual data accumulated, as a result of which two theories were formed concerning the action of electric current on living matter (1) the concentration theory of Nernst (1908) and (2) the condenser theory developed by Hermann (1905), Chagovets (1903), Lapicque (1926), Ebbecke (1927), Hill (1935) and others.

It is known that the stimulus originates at the cathode. It is also known that when current is let through the cell, a change occurs in the concentration of electrolytes at the electrodes. Nernst regarded this as the reason for the physiological action of electric current. When the change in concentration at the cathode reaches a certain threshold value, excitation arises.

The concentrational effect of electrolytes at the membrane during the passage of current depends on two opposite processes—accumulation of ions under the influence of current and their diffusion in the opposite direction. On the basis of these considerations Nernst developed the formula

$$c - c_0 = ki\sqrt{t}$$

where $c - c_0$ is the threshold difference between ionic concentrations necessary for the appearance of excitation, while k is a constant.

Consequently, according to Nernst, on varying intensity and time, the value of $i\sqrt{t}$ should be constant ($i\sqrt{t}$ = constant or i^2t = constant) in order to obtain a threshold stimulation.

The energy (E) released by the current equals the product of the current intensity (i), voltage (v) and the time of passage of current (t).

$$E = i \cdot v \cdot t, \text{ or } E = i^2Rt.$$

In ordinary excitometric systems, on changing the voltage (v), the current intensity (i) changes to the same extent, while the resistance of the circuit remains constant. According to the theory of Nernst, when excitation occurs the magnitude of i^2t should be constant, i.e., under these conditions the amount of energy released by the threshold current should have a constant value.

Further, Nernst had to explain why in the region of currents of long duration the value of the threshold no longer depends on time of passage of the current. Since Nernst himself was not a physiologist, he refers in his work (Nernst, 1908) to a letter from Professor (of Physiology) von Kries informing him that on slow increase of the current the latter may be physiologically inactive. Nernst assumed that on gradual accumulation of ions at the membrane, certain chemical compounds were formed which put into action some kind of adaptation to the excitant by "accommodation" of the membrane to the action of ions. Due to this fact the threshold of excitation should increase. This accommodation is absent with rapid action of current.

336 This part of Nernst's hypothesis is not tenable. It is natural to expect accommodation of a living system to any foreign or harmful agent, but there

is no need for the system to adapt and to become insensitive to a physiologically adequate excitant—i.e., electric current, by which transmission of the impulse occurs along the fibers. Nernst himself gave no evidence in favor of his hypothesis; nevertheless his idea became very popular among physiologists, serving as the basis for the so-called theory of accommodation. This is usually used to explain the lack of significance of duration of action of the stimulating current at sufficiently long time intervals (rheobase).

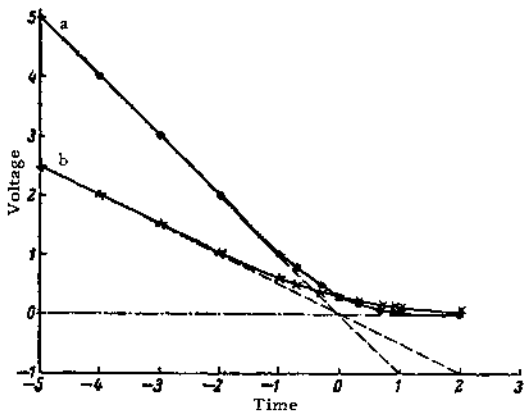


FIGURE 190. Logarithmic voltage-time curves plotted according to the formula

$$i = \frac{a}{t^n} + b$$

a—at a = 1; b = 1, n = 1; b—at a = 1, b = 1, n = 0.5.

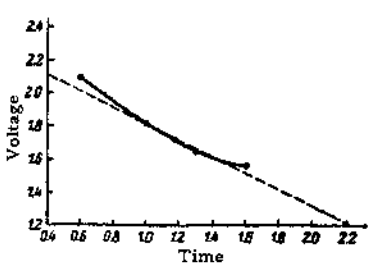


FIGURE 191. Logarithmic voltage-time curve of a frog nerve plotted according to data of Weiss, taken from Nernst (1908)

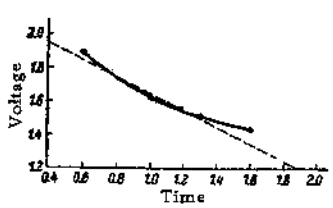


FIGURE 192. Logarithmic voltage-time curve of a tortoise nerve plotted from the data of Weiss, taken from Nernst (1908)

The theory of Nernst which requires a constant $i\sqrt{t}$ on stimulation with electric current obviously contradicts the empirical data of Hoorweg, Weiss, and other authors who have shown, in their studies on vertebrate nerves, that the product $i \cdot t$ is constant. Nernst attempted to prove his point experimentally, as against that of Hoorweg. In his 1908 work he gave a large number of tables mainly taken from other authors, illustrating intensity-time curves for various objects. Using these data, he tried to prove that at a certain approximation the product $i\sqrt{t}$ is always a constant. However, careful analysis of these data makes Nernst's argument very doubtful.

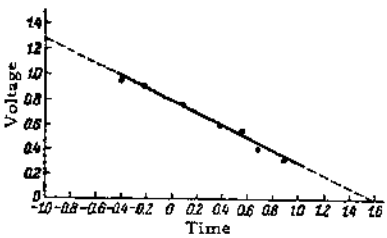


FIGURE 193. Logarithmic voltage-time curve of muscle from the mantle of Aplysia punctata, plotted from the data of Lapicque, taken from Nernst (1908)

It has already been pointed out (p. 274) that in logarithmic presentation the intensity-time curve corresponding to Hoorweg's formula ($i = \frac{a}{t} = b$) acquires the form illustrated in Figure 190 (curve a). Its right wing approaches asymptotically a horizontal straight line parallel to the abscissa at a level equalling log b. The left wing approaches a straight line inclined to the abscissa at 45°, cutting off equal segments from the coordinate axes (1:1).

If Nernst and not Hoorweg is right, and not $i \cdot t$ but $i\sqrt{t}$ is a constant value, then the angle between the straight line (to which the left wing of the logarithmic curve approaches) and the abscissa should be more acute and the straight line should intersect the abscissa forming a segment twice as long as on the ordinate (2:1) (Figure 190, curve b). The left wing of curves a and b approximately corresponds to the formula $i \cdot t$ = constant (curve a) or $i\sqrt{t}$ = constant (curve b). The right wing corresponds to the equation $i = b$. The middle part of the curve smoothly connects the two wings, approaching toward straight line asymptotes. In order to check the validity of this (or another) formula, it is necessary to choose a region sufficiently removed from the rheobase which, on the logarithmic graph, approaches a straight line, and then determine its slope. This was the procedure of Lapicque (1926), Hill (1935), Bugnard and Hill (1935), Rosenberg (1935), Nasonov and Rozental' (1953) and others, when they wanted to check the agreement between empirical intensity-time curves and this formula and others.

Nernst did not do so. In the majority of his examples, the middle segments of the intensity-time curve were used, which were not very distant from the rheobase, and relatively small segments were given where the extreme points differed from each other only 5-10-fold in time. Figure 191 and 192 show logarithmic graphs, according to the data of Weiss, taken from Nernst. From these figures it is seen that the author chose the flexion points of the curves where the tangential line is located, approximately at a slope of 1:2. It is understandable that a small segment of the curve in this region will not differ very markedly from this tangential line. This device was used by Nernst in all cases in which he tried to prove the applicability of his formula to vertebrate nerves. He could not help noticing, however, that the greatest deviations from this formula are observed at the extreme points of the segment chosen by him, both deviations

resulting in values higher than expected. Nernst explained this by the fact that in the region of large intervals, the proximity of the rheobase showed its influence, while the anomalies in the region of small intervals he compared to the known anomalies in physics manifested in the region of action of very high pressures, very low temperatures, etc. In reality, as will be seen from Figures 191 and 192, the point is that he used segments at the flexion point of the curve, where the effect of the rheobase was still
338 felt all along the segment. If Nernst would have taken a segment sufficiently far removed from the rheobase, then the rule corresponding to the equation $i \cdot t$ = constant would have been manifested in its pure form, while the logarithmic curve would have turned into a straight line with a slope of 1:1.

Nernst also studied the muscles of the invertebrate Aplysia punctata, the measurements of excitability of which were taken by him from Lapicque. Figure 193 shows that when the logarithmic form of the curve is used, all the points are arranged nicely for a long distance on one straight line, with a slope of 1:2. In the given case the effect of the rheobase was not detected at all, and therefore the product $i\sqrt{t}$ is constant.

As already stated, the rule that threshold energy is constant is true for the action of current on receptors of nerves and muscles of certain invertebrates, etc. The attempts of Nernst to prove the universality of this rule and its applicability to tissues such as vertebrate nerves and muscles are clearly erroneous.

Somewhat later, after the formulation of Nernst's theory, a number of attempts were made to construct a theory of electric excitation based on the concept of the cell as a condenser. These theories, like that of Nernst, were based on the assumption that the cell is isolated from the medium by a semipermeable membrane, at the inner and the outer surfaces of which a double electric layer should exist, due to the different intra- and extracellular electrolyte content. The presence of this layer presumably endowed the membrane with certain characteristics as a result of which the cell could act as a condenser. Physiologists who developed this theory (Chagovets, 1903; Herman, 1905; Lapicque, 1926; Ebbecke, 1927; Hill, 1935 and others) arrived at a completely arbitrary assumption that moving excitation appeared when the voltage of the charged cellular condenser reached a certain value. At this instant the condenser discharged.

On the basis of the formula of condenser discharge, these investigators reached the conclusion that for the appearance of a moving wave of excitation, a constant amount of electricity ($i \cdot t$ = constant = a) was required, i.e., they theoretically confirmed the data of Hoorweg-Weiss. In contradiction
339 to the requirements of the theory of Nernst, the amount of threshold energy ($i\sqrt{t}$) is not at all constant here. The energy curve has a clearly expressed minimum in its middle part, in the region of intersection of the asymptotes on the logarithmic graph.

However, as with the theory of Nernst, it is impossible to prove the universal applicability of the condenser theory. We have already pointed out that in reality the two theoretical equations for small intervals of excitation—$i \cdot t$ = constant and $i\sqrt{t}$ = constant, are two extreme limits between which are located the tissues with intensity-time curves corresponding to the equation $i \cdot t^n$ = const, where n varies from 0.5 to 1.0.

The data presented earlier forced us to reject the generally accepted idea of the cell as an osmometer surrounded by a semipermeable membrane. We assume that living protoplasm is a coacervate behaving as a water-

insoluble phase system, in which the majority of electrolytes are present in a bound state, while the dissolved electrolytes exist at a different concentration than in the surrounding solution. As shown by Nernst (1892), such a phase should possess a certain capacity at the division boundary and may be looked upon as a condenser. Nernst also showed that on passing an electric current through the boundary between water and the phase system, polarization was observed at this boundary, together with a noticeable change in ionic concentrations, easily detectable by the use of any pH indicator.

Consequently, both condenser and concentration theories are equally acceptable from the point of view of the membrane and the phase theories of cell structure.

Which of these theories is the more tenable?

The theory of Nernst considers excitation to occur as a result of the concentration of electrolytes at the cell surface, altered by the effect of the passing current. The immediate reason for excitation in this case is the chemical action due to the unusual concentration of ions. In this sense Nernst's theory may be considered as relying on chemical laws.

The condenser theory operates with a purely electrodynamic concept of discharge of the condenser, charged to a certain critical potential. The theory does not state specifically how this discharge is transformed into an excitation wave. In this respect Nernst's theory should be given priority, being more factual.

As we have seen, however, experimental data connected with analysis of the intensity-time curve for different tissues do not confirm either this or the other theory, and leave open the final solution of the problem.

Recently, however, a quite unexpected possibility presented itself for solution of this problem in terms of Nernst's theory, but from another standpoint, i.e., in connection with studies on the effect of interelectrode distances on the electric stimulation of the nerve (Nasonov, 1955). We shall now pass on to the presentation of these data and the conclusions drawn from them.

Effect of Interelectrode Distance on Excitability Threshold

The relationship between excitability threshold of the nerve and the distance between the electrodes was extensively studied for the first time by Chir'ev (1877), and simultaneously, and probably independently, by
340 Marcuse (1877)*. Both authors found that on increasing the interelectrode distance, the excitability threshold of the nerve gradually diminished, approaching a certain constant value when a distance of 1-2 cm was attained. This observation was confirmed by Cardot and Laugier (1914, Cardot, 1914), who described a decrease in the rheobase and simultaneous increase in chronaxy on removal of the electrodes from each other. Later, Keil (1922), Eichler (1929), and Schriever (1932) showed that on increase of the interelectrode distance the threshold voltage at first diminished and subsequently increased.

Later, the decreased excitability threshold of the nerve to a certain level with increase of interelectrode distance was again confirmed by using a more detailed method (Rushton, 1927, 1934), with experiments on a

---

* For earlier literature see Schriever and Bürkner (1940).

medullated frog nerve, and by Rosenberg (1935) on a nonmedullated nerve of the crab Maja. Thus, decrease in excitability threshold of the nerve on moving the electrodes away from each other may be considered as a confirmed fact. However, it is not easy to explain this fact, since while moving the electrodes apart, one may expect only a decrease in intensity of the stimulating current, which should cause not a decrease but an increase in the threshold level. However, in the case of a stabilized intensity of current, the distance between the electrodes should not affect the threshold at all.

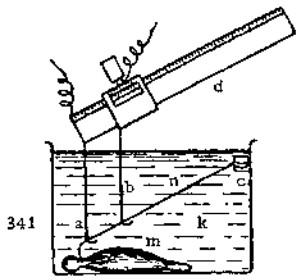


FIGURE 194. Diagram of experimental chamber for altering interelectrode distance

a—fixed electrode; b—electrode moving along the nerve (n) by the use of a micrometer screw on a scale (d) supplied with a vernier; k—chamber filled with vaseline oil; c—glass rod; m—muscle.

In order to determine the excitability threshold of the nerve, we (Nasonov, 1955) used condenser discharges from the instrument described above (Figure 166). The change in distance between the electrodes was carried out by means of the apparatus described in Figure 194. The calf leg muscle of a frog was placed at the bottom of a glass chamber, and its nerve placed over a fixed silver electrode (a) serving as the cathode and over a moving anode (b). It was directed at an angle of 45° to the upper part of the chamber, where a section of the spinal cord was placed on a glass rod (c). The electrode (b) moved by the aid of a micrometer screw on a scale (d) supplied with a vernier, and was placed at the same angle as the nerve. The whole chamber was filled with vaseline oil in order to eliminate drying-out of the preparation.

The sloping position of the nerve in vaseline oil, and the corresponding sloping movement of the electrode, assured the tautness of the nerve and the ideal gliding of the electrode on its lower surface at a certain constant contact.

The experiments were performed in May and June of 1953 on nerve-muscle preparations of a frog (Rana temporaria) in the above-described chamber, with ordinary silver electrodes. The distance between the electrodes varied within the range of 1-30 mm. At different arrangements of the moving electrode, measurements of excitability thresholds were taken consecutively with 5 condensers of different capacities (90, 1, 0.1, 0.01 and 0.001 microfarad). Excitation was carried out according to the scheme in Figure 195, r_2 being 100 ohm.

Results are shown in Figure 196. Only at high capacities (90 microfarads) and the prolonged discharges connected with them, a curve similar to that obtained by other investigators was obtained by us. On increasing the distance between the electrodes, the excitability threshold decreased at first, reaching a minimum at a distance of approximately 10 mm. Further on, it remained almost constant and only after considerable distances from the other electrode it again increased somewhat. At a lower capacity, and consequently at a shorter time of discharge (Figure 196; 1 microfarad, 0.04 milliseconds), the nature of the curve changed. Minimal decrease in threshold was attained when the electrodes were approximated (to about 5 mm), and a more marked increase in the threshold appeared after reaching

the minimal distance. On further decrease of the capacities, the initial drop in threshold level on removal of the electrodes became less and less noticeable, while the rise of the right hand part of the curve increased all
342 the time*. It is interesting that this increase in the threshold was not rectilinear, consequently, its increase was not directly proportional to the length of the segment but related to it by a certain exponential function. It will be seen later that this fact is of a certain theoretical interest.

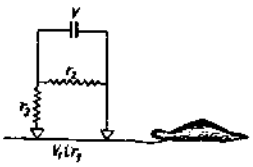


FIGURE 195. Diagram of apparatus used for excitation in experiments with alteration of interelectrode distances (according to Nasonov, 1955)
343 For explanation see text.

In order to explain the obtained data, we assume that the different nature of the curves for different durations of discharges may be explained by the fact that with metallic electrodes, more prolonged discharges are accompanied by greater polarization on the electrodes, exerting an additional, external resistance in relation to the nerve (r_3 in Figure 195).

In order to confirm this hypothesis, we first plotted the curve of relationship between the threshold voltage and interelectrode distances for short discharges, in the absence of resistance in the external circuit (Figure 197, A, 1). Parallel to this, similar curves were obtained with the same nerve, using a switch and introducing a rising resistance (Figure 197, A, 2-4). The same was done with respect to duration of discharges (Figure 197, A, 5-7). We assumed that if the differences between the curves obtained can indeed be explained by the magnitude of external resistance (r_3) caused by polarization, then by introduction of a sufficiently high external resistance the differences between the curves should be evened out, and approach in their form the extreme curve corresponding to discharge of longest duration (90 microfarads).

Our assumptions were fully confirmed. Indeed, with increase in external resistance (r_3) the initial decrease in threshold voltage became more marked and more protracted, and the subsequent increase became more gradual. As a result of this, the curves for short term impulses (Figure 197, A, 2-4) became more and more similar to those of long term impulses (Figure 197, A, 5-7).

As far as the curves for 90 microfarads are concerned, introduction of resistance made the decrease in threshold level with removal of the electrodes more marked. Further, after reaching minimal levels, the threshold remained constant until the end, within limits of error (Figure 197, A, 7). This extreme curve corresponds exactly to the curves for relationship between threshold and interelectrode distance as described by other authors.

Substitution of silver electrodes by nonpolarizing DuBois-Reymond electrodes led to the same results even without introduction of any additional resistance into the circuit (Figure 197, B). This was due to the fact that, on the one hand, nonpolarizing electrodes themselves possess a very high

---

* Most of our experiments were performed on a nerve of Rana temporaria. A series of curves was obtained using a larger frog, Rana ridibunda, in which the experiments could be carried out with a nerve segment approximately 2.5 cm long between the muscle and the nearest branching of the nerve. The results obtained were similar to the previous ones.

resistance (about 80 k/ohm) and, on the other hand, polarization which is, as we have shown, the main reason for the difference between curves of various capacities, was reduced to a minimum. In some cases in which the DuBois-Reymond electrodes were used, a small divergence between the curves for low and high capacities was also observed. In other cases, when polarization on the electrodes was completely eliminated, both curves practically coincided.

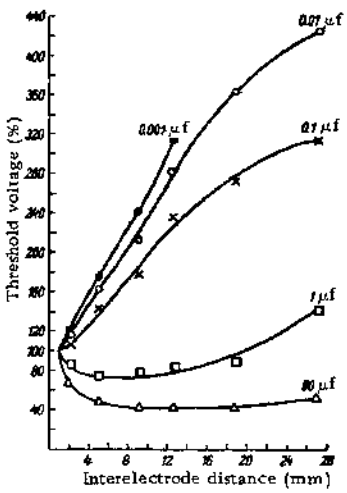


FIGURE 196. Nerve-muscle preparation of frog. Relationship between threshold voltage and interelectrode distance at different durations of the excitatory stimuli (according to Nasonov, 1955). The threshold voltage at 1 mm distance between the electrodes was taken as 100%.

From the above the following conclusions can be drawn:

1. On increase of the interelectrode distance the excitability threshold of the nerve at first decreased, and began to increase again after reaching a certain minimum.

2. This increase was not rectilinear (especially well seen on curves of small capacities).

3. Introducing external resistance (r_3 in Figure 197, A), or creating resistance as a result of polarization on metallic electrodes and in the nerve on prolonged discharges, caused a shift in the position of the minimum on the curve to the region of greater interelectrode distances.

4. At a sufficiently high external resistance (r_3) the increase in threshold along the investigated nerve may be indiscernible by the method used (Figure 197, B, 2). In such cases a curve of relationship of the threshold to the interelectrode distance was obtained, as has been repeatedly described by previous investigators.

5. On the basis of all that has been said, an explanation for the increase in chronaxy on moving the electrodes away from each other may be as proposed by Cardot and Laugier (1914):

$Chr = \frac{a}{b}$ where 'a' is the excitability threshold of the nerve on sufficiently short current impulses, while b is the rheobase, i.e., the excitability threshold
344 with sufficiently prolonged current pulses. Figures 196 and 197, A show that with increase in the interelectrode distance the ratio $\frac{a}{b}$ increases, which should inevitably lead to increase in chronaxy. Thus the fact discovered by Cardot and Laugier is the result of partial polarization at the electrodes. It is quite obvious that in the case corresponding to Figure 197, B, there can be no increase in chronaxy on moving the electrodes away from each other.

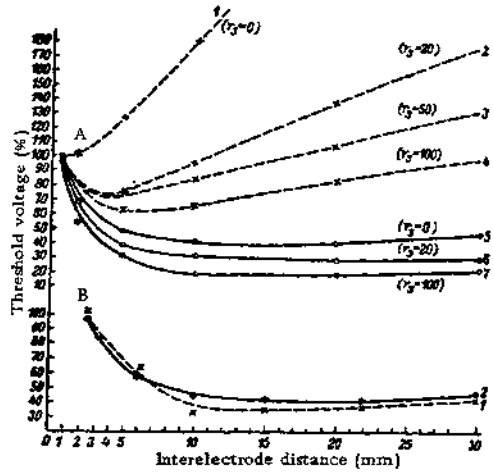


FIGURE 197. Nerve-muscle frog preparation. Relationship between threshold voltage and interelectrode distance, with different additional resistances (r_3 in the circuit) (according to Nasonov, 1955)

A—change in excitability on increasing r_3 from 0 to 100 k ohm: 1-4—for excitatory stimuli of a duration of 0.02 microfarads, 5-7—same, duration of 90 microfarads; B—change in excitability using nonpolarizing electrodes: 1—for excitatory stimuli with a duration of 0.01 microfarad, 2—same, duration of 90 microfarads. The threshold voltage at a minimal distance between the electrodes was taken as 100%.

Importance of Voltage, Intensity and Energy of Excitatory Current for Appearance of Excitation in the Nerve Fiber

Thus, we have seen that with increase in distance between the electrodes the excitability threshold drops at first, increasing later. In order to understand this bi-phasic relationship we must first see how the threshold would change if it depended only on the voltage or on current intensity.

Figure 195 shows the manner in which the excitation was carried out: v is the voltage of the discharged condenser, v_1—voltage on the excitatory electrodes, i—intensity of current passing through the nerve, r_1—resistance of the nerve segment, r_2—resistance of the shunt, r_3—external resistance of the circuit.

I. Let us assume that the onset of excitation is determined only by the voltage on the electrodes (v_1) but does not depend on the intensity of the passing current. On the basis of the scheme (Figure 195)

$$\frac{v}{v_1} = \frac{r_1 + r_3}{r_1} \quad v = v_1 \frac{r_1 + r_3}{r_1},$$

and since according to our assumption v_1 should be constant,

$$v = \text{const} \frac{r_1 + r_3}{r_1}.$$

This formula determines the relationship of the threshold voltage v in such a case to the distance between the electrodes (assuming that the resistance of the nerve segment r_1 is proportional to its length l). A group of such theoretical curves is illustrated in Figure 198, A, for various values of r_3*. If $r_3 = 0$, the threshold voltage does not depend on the distance between the electrodes. If, however, the resistance is increased more and more ($r_3 = 1, 5, 100$) curves are obtained which show a decrease in the threshold value. Subsequently the latter approaches a certain constant value ($v = \text{const} = v_1$). These curves are somewhat similar to curves obtained experimentally for a high r_3. However, they differ from the empirical ones in that no matter how much the value of r_3 diminishes, the curves cannot increase with increased interelectrode distance.

II. Let us now assume, as is done by the majority of physiologists, that the onset of excitation is determined by intensity of the passing current, but does not depend upon its voltage on the electrode.

We have seen that

$$v = v_1 \frac{r_1 + r_3}{r_1}$$

345 or

$$v = i \cdot r_1 \frac{r_1 + r_3}{r_1} = i(r_1 + r_3);$$

and since according to our assumption i should be constant,

$$v = \text{const}\ (r_1 + r_3).$$

This equation shows the dependence of threshold voltage on the distance between the electrodes (l or r_1) under the condition that this threshold is determined only by the intensity of current. Such curves, for different values of r_3 (from zero to 1,000) are illustrated in Figure 198, B. With a very high value of r_3 it will be seen that the intensity of the current is practically stable and therefore the threshold voltage almost does not depend on the interelectrode distance. On consecutive decrease in r_3, a series of rectilinear increasing curves of the threshold is obtained. The smaller is r_3, the steeper will be the increase in the threshold value with increase in interelectrode distance. These curves also do not agree with those obtained in practice. In the latter, the increase of the distance between the electrodes

---

* In Figure 198, as in the previous curves, the threshold voltage corresponding to $r_1 = 1$ mm is accepted as 100%.

can under no circumstances lead to a decrease in threshold. They only resemble empirical curves with low values of r_3, differing from the latter by a rectilinear increment of the threshold voltage. In the experimental curves this increase, as mentioned above, is always curvilinear.

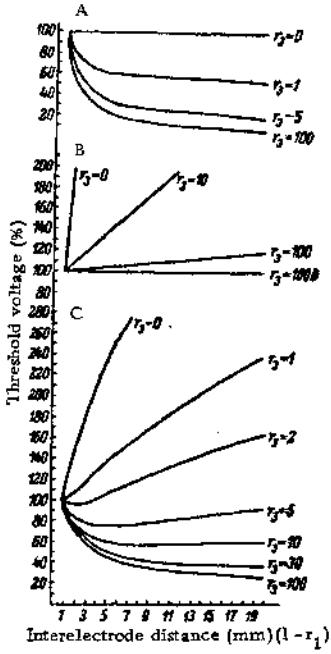


FIGURE 198. A group of theoretical curves of relationship between the threshold voltage and distance between the electrodes at different values of external resistance in the circuit (according to Nasonov, 1955).

A—assuming that the threshold voltage is determined by the voltage of excitatory current; B—assuming that the threshold voltage is determined by the intensity of the current; C—assuming that the threshold voltage is determined by the energy of current.

Thus, the experimental curves do not fully correspond either to the first or to the second hypothesis. Initially, and with a high external resistance (small r_1 and large r_3), they resemble the curves obtained with a large voltage, while finally and at a small external resistance (large r_1 and small r_3) they correspond to the dependent relationship between the intensity of the current and the onset of excitation.

III. In order to solve this problem one more hypothesis is required, namely, that the onset of excitation in a nerve fiber is determined neither by the voltage at the excitatory electrodes nor by the intensity of current, but by the energy released by the current in the excited segment of the conductor. This energy $E = v_1 it$, where t is the time of passage of current. It is known that $v = v_1 \frac{r_1 + r_3}{r_1}$ or $v_1 = \frac{vr_1}{r_1 + r_3}$.

At the same time $v = i(r_1 + r_3)$, from which it follows that $i = \frac{v}{r_1 + r_3}$ and consequently,

the energy $E = v_1 it = \frac{v^2 r_1}{(r_1 + r_3)^2} \cdot t$, or

$v = \sqrt{E} \frac{r_1 + r_3}{\sqrt{r_1}} \frac{1}{\sqrt{t}}$. Since according to our assumption the threshold energy E should be constant ($\sqrt{E} = K_1$), then

$$v = K_1 \frac{r_1 + r_3}{\sqrt{r_1}} \frac{1}{\sqrt{t}}. \qquad (1)$$

If the resistance in the circuit is constant $\left(\frac{r_1 + r_3}{\sqrt{r_1}} = K_2\right)$ and only the time of passage of current changes, the equation of Nernst will be obtained, relating the threshold voltage to the time of passage of current at a constant resistance in the circuit. Indeed, equation (1) will acquire the form $v = K_1 . K_2 . \frac{1}{\sqrt{t}}$

or $v^2 \cdot t = K$. But we are interested in the relationship between threshold voltage and resistance at a constant time interval ($\frac{1}{\sqrt{t}} = K_3$). Under such conditions equation (1) will acquire the form

$$v = K_1 K_3 \frac{r_1 + r_3}{\sqrt{r_1}} = \text{const} \frac{r_1 + r_3}{\sqrt{r_1}} \qquad (2)$$

Thus the threshold voltage should depend on the distance between the electrodes, where the excitation appearing in the nerve depends on the energy released by the excitatory current. Such curves for different values of r_3 are illustrated in Figure 198, C.

We shall analyze this equation. With small values of l or r_1 (considerably smaller than r_3) the numerator of the right-hand part of the equation is close to a constant value; consequently, the threshold voltage is inversely proportional to the square root of the interelectrode distance. Further, as r_1 approaches r_3 in its value the curve drops to a certain minimum, after which it begins to rise. When r_1 exceeds the value of r_3 to such an extent that the latter can be disregarded, equation $v = \text{const} \frac{r_1 + r_3}{\sqrt{r_1}}$ will be transformed into the following: $v = \text{const} \frac{r_1}{\sqrt{r_1}} = \text{const}\sqrt{r_1}$. In other words, the threshold voltage will increase in direct proportion to the square root of the interelectrode distance. Consequently, this increase will be rectilinear.

From the equation $v = K\frac{r_1 + r_3}{\sqrt{r_1}}$ one can determine when the dependence

347 curve of the threshold voltage on the interelectrode distance will reach minimum. Consequently this equation may be transformed as follows:

$$v = K\left(\frac{r_1}{\sqrt{r_1}} + \frac{r_3}{\sqrt{r_1}}\right) = K\left(\sqrt{r_1} + \frac{r_3}{\sqrt{r_1}}\right). \qquad (3)$$

It is obvious that on increase of r_1, the first term of the binomial ($\sqrt{r_1}$) will increase while the second term ($\frac{r_3}{\sqrt{r_1}}$) will decrease. The minimum value of the function will take place when $r_1 = \frac{r_3}{\sqrt{r_1}}$ or when $r_1 = r_3$. In other words, the minimal value of the threshold voltage is achieved when the internal resistance of the segment of the excited nerve (r_1) becomes equal to the external resistance of the circuit (r_3). It follows from this fact that on increase of r_3 the minimum should shift in the direction of greater interelectrode distances. This purely theoretical conclusion may be checked experimentally.

If a series of experimentally obtained curves is now compared (Figures 196 and 197, A) with the theoretical curve (Figure 198, B) it will be seen that the similarity between them is striking. In both cases an initial decrease is noticed in the threshold value with an increase in distance, followed by an increase which subsequently continues in curvilinear fashion.

Further, in both cases the increase in external resistance (r_3) has the same effect. With increase in r_3 the phase of decrease in v becomes more prominent and prolonged, and at a sufficiently large r_3 the level of v at a certain interval of r_1 may be assumed for all practical purposes to be constant (compare Figure 197, A, 7, with Figure 198, B, r_3 = 100). Finally, it is seen that in complete agreement with the requirements of the theory, the position of the minimum on the experimentally obtained curves shifts with increase in external resistance (r_3) to the region of greater interelectrode distances (Figures 197, 198, B). All this leads to the conclusion that on electric excitation the decisive condition for onset of excitation in the nerve fiber is the energy released by the current, and not the amount of electricity, as is assumed by the majority of physiologists.

Previous authors usually explained the decrease in threshold, after increasing the interelectrode distance, by stating that when the electrodes are brought closer to each other than 1 cm, the opposite poles somehow mutually weaken their effect on the tissue. This explanation is not very convincing, since it does not explain the nature of this neutralization.

Another explanation was given by Beritov (1947), who assumed that with a small interelectrode distance a droplet of Ringer's solution forms, resulting in a short circuit. This explanation is also untenable, since in our experimental conditions with use of vaseline oil, there are no water droplets. In addition, Beritov maintained that a decrease in threshold is observed only at an interelectrode distance not exceeding 2-3 mm, while in reality it can easily be observed in an ordinary nerve-muscle preparation even at a distance of 10 mm. Rushton (1927, 1934) who worked with a nerve of the large American frog, observed a drop in threshold even at an interelectrode distance of up to 20 mm. It is self-evident that a short circuit through a droplet between the electrodes cannot occur here, especially when working in a moist chamber.

A third, most elaborate but somewhat speculative explanation, was
348 given by Rushton (1927, 1934). He started from two hypotheses stated in terms of the generally accepted membrane theory. First he assumed that a medullated nerve fiber is a good conducting core surrounded by a poorly conducting sheath (probably myelin). This fiber is surrounded by a good conducting layer of Ringer's solution. The current penetrates easily into the intercellular space and with greater difficulty inside the fiber, through the myelin.

The second assumption was that excitation arises on the cathode only when current leaves the fiber across the myelin sheath. Current, entering the nerve from the anode, divides into two sections. The main one is directed along the intercellular space toward the cathode, while the other penetrates across the poorly-conducting myelin sheath into the well-conducting nerve fiber, moving along the latter and secondarily passing across the sheath, leaving the fiber under the cathode (Figure 199). Thus, the fraction of current passing through the fiber overcomes the resistance of the contents of the nerve fiber, which is proportional to the length of the segment between the electrodes plus twice the resistance of the myelin sheaths. If the distance between the electrodes is short, the resistance of the sheath is relatively high, and the fraction of the current moving inside the fiber becomes smaller as compared with the fraction directed into the intercellular space, due to which the value of the threshold current intensity is relatively high. With increase in interelectrode resistance the resistance

of the intercellular space and the nerve contents increases in proportion to this distance, while the double resistance of the sheath remains the same, due to which the resistances overcome by both fractions of the current approach each other in value. As a result, a relatively greater fraction of current passes inside the fiber, causing a drop in the threshold on increasing the distance between the electrodes.

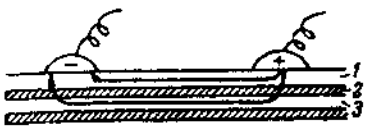


FIGURE 199. Diagram of distribution of current branches passing through the nerve (according to Nasonov, 1955)

1—space between the fibers;
2—sheath of the fiber;
3—axis cylinder.

This is the explanation of the facts given by Rushton. As already mentioned, this explanation sounds somewhat artificial. The postulates on which this explanation is based seem to us to be erroneous. First of all, the author's attempt to explain increase in excitability on increasing the distance between the electrodes by the properties of distribution of the current in a medullated nerve is not correct, since Rosenberg (1935) observed the same phenomena also on a nonmedullated nerve of the crab Maja.

More recently the effect of interelectrode distance on the value of the threshold was studied by Rozental' and Shapiro (1956) on a nonmedullated nerve of the crab Hyas coarctatus. The results of their
349 work are illustrated in Figure 200. Each point on this graph represents an arithmetic mean from 14 experiments, and the curves given are calculated according to the formula $v = K\frac{r_1 + r_3}{\sqrt{r_1}}$ It can be seen that the empirical data agree well with those theoretically expected.

Schriever and Bürkner (1940), in our opinion, gave an incorrect explanation of the relationship of threshold potential to the interelectrode distance. These authors start from the well-known but purely hypothetical concept of the nerve fiber as a good conductor surrounded by a semipermeable insulating membrane possessing a certain capacity. When the latter is charged to a certain potential, excitation occurs. On the basis of this scheme the authors arrive at the following, quite complicated formula:

$$v = v^1 \frac{R(W_H + W_1 + W_K + W_2) + W_H(W_1 + W_K + W_2)}{W_H(W_1 + W_K + W_2)},$$

where v is the threshold potential, v^1—the potential on the electrodes, R—the external resistance of the circuit (our r_3), W_H—the resistance of the membrane, W_K—the resistance of the fiber core, W_1, W_2—the resistance of shortcircuiting the membrane. It can be shown that with increase in the interelectrode distance this function at first decreases, increasing subsequently, i.e., it corresponds, as it were, to what really takes place. However, it is not difficult to see that the formula of Schriever and Bürkner does not reflect the actual state. If R in this formula is given a value of zero, the formula will acquire the form of $V = V^1$. In other words, under these conditions the threshold potential becomes a constant value independent of interelectrode distance. It is, however, known that when $R = r_3 =$ zero,

the threshold potential increases maximally when the electrodes are moved apart (Figure 197, A, 1). Thus, the explanation suggested by Schriever and Bürkner is wrong.

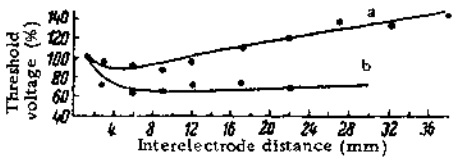


FIGURE 200. Nerve-muscle preparation of a crab. Relationship between threshold voltage and interelectrode distance (according to Rozental' and Shapiro, 1956). The curves were calculated according to the formula $v = K\frac{r_1 + r_3}{\sqrt{r_1}}$. The points represent experimentally obtained data; a—without resistance (average from 29 experiments); b—with a resistance of 20 k ohm (average of 14 experiments). The threshold voltage at a distance between the electrodes of 1.5 mm is taken as 100%.

Experiments with Isolated Fibers

As we have seen, Rushton's theory is based on the distribution of electric current between the fibers and intercellular spaces. Consequently, with a tissue in which the possibility of short-circuiting the current through the connective tissue layers is excluded, there should be no increase in the threshold on bringing the electrodes closer. A single nerve or muscle fiber is such a tissue, and experiments performed with it should verify Rushton's theory or otherwise.

Certain indications of such experiments are found in the study of Hodgkin (1938) carried out on a single nerve fiber of the crab Carcinus. Unfortunately, the author gives neither numerical data nor graphic illustrations of this point, limiting himself only to a few lines in the text. On page 104 he indicates a fact, in his opinion quite important, that on decrease of the interelectrode distance the threshold current increases, indicating decrease in excitability. Repetition of these experiments, and a more convincing confirmation of the results obtained by Hodgkin would be direct confirmation of the error of Rushton's explanation, and of the correctness of our assumptions that the
350 decisive factor in the onset of excitation is the energy of the stimulating current and not the amount of electricity.

In view of the fundamental importance of this question, similar experiments were designed in our laboratory by Krolenko (1956), using isolated muscle fibers of the tetanic bundle m. ileofibularis of a frog. The whole length of the fiber was isolated. At one end of the fiber the shreds of neighboring fibers were thoroughly removed, so that its junction with the tendon, and part of the tendon itself, were thoroughly cleaned. Serfin* was attached to the tendon at this end of the fiber, by the aid of which the fiber was attached to a fixed silver hook; a silver weight of 1-1.5 mg was attached to the opposite end of the fiber.

The attached fiber was thus freely suspended in a chamber with flat-parallel glass walls (Figure 201, k) fixed by the moving-stage on a horizontally placed microscope (Figure 201, m). The fiber was illuminated by a source of light (Figure 201, o) and the image of the fiber end with the weight was thrown by the microscope onto the screen (Figure 201, s).

---

* [A certain synthetic substance.]

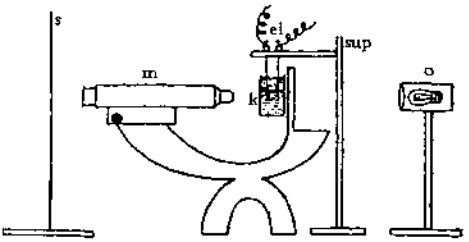


FIGURE 201. Diagram of apparatus for excitation and recording of contractions of an isolated muscle fiber of a frog (according to Krolenko, 1956)

m—microscope in horizontal position; k—chamber with flat-parallel walls mounted on the microscope turntable by the moving-stage; el—electrodes, on one of which the fiber was suspended; sup—support; o—illumination; s—screen.

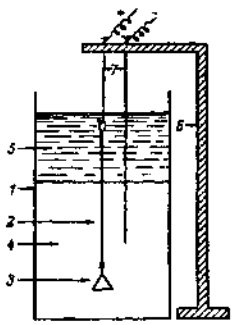


FIGURE 202. Chamber used in experiments with an isolated nerve-muscle preparation of a frog (according to Krolenko, 1956)

1—chamber; 2—fiber; 3—weight; 4—Ringer's solution; 5—vaseline oil; 6—support; 7-7—electrodes.

The chamber was filled with Ringer's solution, on the surface of which a layer of vaseline oil was placed (Figure 202). The hook on which the fiber was hung served as one of the electrodes for stimulation of the fiber. The other electrode consisted of a silver wire immersed in Ringer's solution. The actual site of application was the tendon, while the cathode was the boundary between the water and vaseline oil. Thus, the value of the interelectrode distance was determined by the length of the muscle fiber segment immersed in vaseline oil. By the use of a micrometric screw, the chamber could be lifted and lowered while the fiber was suspended in a fixed position. In this manner, changes in the interelectrode distances were introduced with a precision of 0.25 mm.

Stimulation of the fiber was carried out by discharges of a condenser of 450 microfarad capacity with a 100 ohm shunt. The threshold was determined by contraction of the fiber, which was recorded visually by oscillation of the image of the weight on the screen (Figure 201, s).

Using the same fiber, the relationship between the threshold of excitability and interelectrode distance was studied at the following resistances, introduced in series—0, 1, 4 and 11 mg ohm. The results of the experiments were unequivocal. One of them is illustrated in Figure 203.

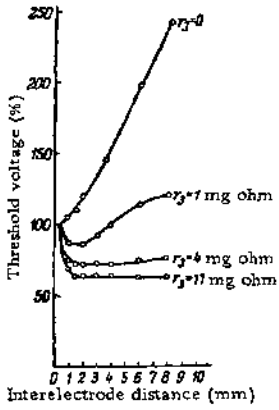


FIGURE 203. Relationship between threshold voltage of an isolated muscle fiber of a frog, and the interelectrode distance, with different additional resistances (r_3) in the 352 circuit (according to Krolenko, 1956). The threshold at a distance of 0.5 mm between the electrodes is taken as 100%.

Comparing this figure with Figures 196, 197 and 198, B, it is easy to see that the same regularity is manifest in all cases. As in those figures, here too, with increase of the interelectrode distance the threshold voltage curve decreases at first, beginning to rise after having passed through a minimum. The position of the minimum in this case, too, depends on the value of external resistance (r_3) introduced in series. The larger the resistance, the more the minimum shifts into the region of larger interelectrode distances. All these facts agree well with our theory of the decisive role of energy released by the stimulating current for the appearance of excitation. But this, also, completely excludes Rushton's explanation based on short-circuiting of the current through the intercellular spaces.

Discussion

In conclusion, we would like to put forward certain ideas in connection with the above data.

At the beginning of this chapter it was pointed out that usually in studies of nerve excitability, a sufficiently high external resistance is introduced into the circuit, so much exceeding the resistance of the nerve itself that its fluctuations should not greatly affect the intensity of the passing current. Such a measure is entirely in order and logical, if it is considered that only the intensity of the current determines the onset of excitation, as has indeed been accepted by the majority of investigators. We came to the conclusion that the decisive factor for the onset of excitation is not the intensity, but the energy released by the current, energy which should be stabilized and protected as far as possible from the effect of changes in the resistance of the nerve. Figure 197, A shows that for this purpose it is first necessary to introduce a high external resistance into the circuit (50-100 k ohm) and secondly, to increase the internal resistance (r_1) to a sufficient extent, moving the electrodes a considerable distance apart (1-2 cm).

Figure 197, A, 7, and 198, C (lower curve) shows that under such conditions there is a region where the alterations in resistance of the nerve (r_1) affect the value of the threshold voltage to a slight extent only. It is obvious that the most convenient distance between the electrodes in this respect will be such a distance at which a minimum excitation threshold is achieved. It has been shown that the minimum threshold of excitation will occur on moving the electrodes apart, when the internal resistance of the interelectrode nerve segment (r_1) will be equal to the external resistance in the stimulating circuit (r_2). By failing to apply the two precautions, an error may occur on determining excitability in those cases when during the experiments the resistance of the nerve is known to change; for example,

in estimating the effect of temperature on excitability, effect of insufficiency or excess of salts, etc.

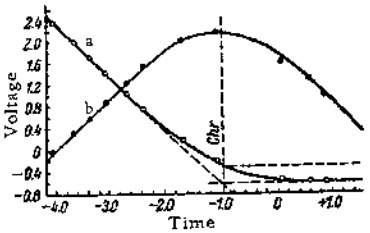


FIGURE 204. Logarithmic voltage-time curve of (a) an autumn frog nerve and (b) excitability curve calculated according to the formula $b = \frac{1}{i^2 t}$ for all the points of this curve (according to Nasonov, 1955)

n(steepness factor) = 1.
Chr—chronaxy.

The conclusion as to the decisive role of energy released by the current, and not of its intensity, in the onset of excitation, forces us also to revise the problem of quantitative evaluation of excitability. It is obvious that for this purpose it is not the reciprocal value of threshold voltage which should be used, as is usually done, but the square of this value; since by increasing the voltage so many times, the intensity of the current usually increases to the same extent, and consequently the energy E at the same duration, increases to the second power. This may be of importance in those cases where an accurate quantitative evaluation of nerve excitability is essential, and not merely the establishment of the fact of its increase or decrease.

If our observations and conclusions are correct, there is a constant for the threshold energy of the current for one and the same time intervals. Within this interval, in order to obtain excitation, the intensity of the current may be changed at will at the expense of its voltage, and vice versa, as long as its threshold energy remains constant. It would seem that this law of constancy of threshold energy should also be valid when intensity of the current is increased, with a corresponding decrease of its duration*. Such a constancy of energy, as we have stated, was postulated by Nernst on the basis of purely theoretical considerations, connected with the laws of accumulation of ions on the surface of cells with passage of current. However, these requirements of the theory were not always confirmed in practice. It was indicated earlier that only for certain tissues at sufficiently short time intervals Nernst's equation ($E = i^2t$ = const) is experimentally confirmed
353 (muscles and nerves of certain invertebrates, smooth muscles of the frog stomach, chloroplasts of Spirogyra, the leg of Vorticella, etc.). In the case of other tissues, for example, muscles and nerves of vertebrates, the formula of Hoorweg-Weiss is true, from which for short time intervals not a constancy of the threshold energy (E) but a constancy of the threshold amount of electricity—Q = it = constant, follows.

It has been shown (Cole, 1933, Colle, 1933, Nasonov and Rozental', 1953) that all cases of dependence of current intensity on time of action may be expressed by the empirical formula $i = \frac{a}{t^n} = b$, where n may acquire values from 1 (Hoorweg, Weiss) to 0.5 (Nernst).

What then is the law on which the action of electric current on a tissue is based?

As already mentioned, our data lead us to the conclusion that within the same time intervals the threshold energy has a constant value. This

---

* Let us remember that the energy of electric current $E = vit$, or $E = i^2Rt$.

conclusion (for currents of the same duration) fully agrees with Nernst's theory. For different time intervals the threshold energy, it seems, should also be constant. However, this may take place only under one essential condition—that the excitability of the fiber does not depend on the duration of passage of the current or on its intensity. In any case, in relation to constant current it is known that during a sufficiently long passage of the current, the excitability of the nerve starts to drop (cathodic depression of Verigo); consequently, this condition is not observed.

We had the opportunity to determine nerve excitability at threshold current of different durations. As just stated, the true excitability of the nerve should be determined by a value reciprocal to the threshold energy. Taking as an example the experimentally obtained intensity-time curve of a frog nerve (Figure 204, a), for each point of it we shall calculate the excitability of the nerve according to the formula $B = \frac{1}{i^2tR}$, where B is excitability, i—threshold intensity, t—time of action and R—resistance, a constant value under the given conditions. Hoorweg pointed out that the threshold energy of a frog nerve on diminishing the time of passage of the current and increase in its intensity has a minimal point. Accordingly, nerve excitability calculated from the threshold energy of the stimulating current passes through a maximum, as can be seen in Figure 204, b. This maximum of excitability corresponds to a region of time-intervals close in value to the duration of the rising part of excitation wave of the frog nerve*. In this
354 region both the time of action and the intensity of the stimulating current are adequate for the intensity and duration of the current of action of this nerve**.

On the above basis it may be assumed that all the known cases of action of electric current on tissue are based on the law of constancy of threshold energy of Nernst: i^2t = const. Deviations from this rule are due to the fact that nerve excitability does not remain constant at different durations of current, but may pass through a maximum in the region of time intervals closest to physiological ones.

---

* It can be shown that the maximum of excitability, and consequently the minimum of threshold energy, coincides with chronaxy in case the tissue under study obeys the formula of Hoorweg: $i = \frac{a}{t} + b$. If in Hoorweg's formula, as it often happens, t has an exponent $n < 1$ the minimum energy does not coincide with chronaxy.

** In the case of tissues which obey the formula of Nernst ($i = \frac{a}{t^{0.5}} + b$) there is no maximum of excitability. With increase in time of action of the current, excitability merely decreases. In the previous chapter (p. 285) it has been pointed out that this fact may be the reason for the seemingly paradoxical possibility that on testing with sufficiently short impulses of current, striated muscles may be less sensitive and "slower" (using Lapicque's terminology) than smooth muscles.

Chapter 2. Electrotonus and Accommodation

Electrotonus of Pflügler

Current physiological textbooks often contain two contradictory and mutually exclusive statements, which may at the same time not appear to be irreconcilable.

These are (1) Pflügler's electrotonus and (2) so-called accommodation, a well-known phenomenon, recently given a detailed theoretical basis by Hill (1935, 1936). According to the first, immediately after passage of a constant current through a nerve, the excitability of the nerve increases at the cathode. According to the second, accommodation of the nerve to the action of constant current consists in the fact that, immediately after passage of constant current, excitability of the nerve at the cathode progressively diminishes.

Hill repeatedly indicated this contradiction, but did not offer any satisfactory solution. In his 1935 paper he writes (p. 119): "Actually 'normal' accommodation is not the usual state of affairs. More often a decrease in threshold at the cathode takes place, as described by Pflüger. However, consideration of this fact would lead to an impossible complication of the equations. Since under certain conditions (for example, a frog nerve at a temperature of about 4°C), electric current only slightly affects excitability, we shall discuss only the case of 'normal' accommodation".

Later, in a paper published in 1936 he writes (p. 346): "Our discussions completely ignore the electrotonic changes of nerve excitability. Thus, experimental data will not tally with our formulas since electrotonic changes may occur during the experiment and distort the entire picture". It may be that electrotonic and accommodational changes at the cathode are entirely different phenomena, and that the former may be deficient or total-
355 ly absent when the latter take place. It seems to us that these considerations of Hill may be regarded more as a refusal to discuss the contradiction rather than a serious solution of the problem. It is well known that under ordinary laboratory conditions both a typical Pflüger electrotonus and a typical Hill accommodation may simultaneously be observed in one and the same nerve. This will be demonstrated by a number of specific examples. At the same time, nerve excitability at the cathode cannot both increase and decrease simultaneously, therefore other solutions must be sought to explain this phenomenon.

It is of interest that a complete and, in our opinion, exhaustive solution of this contradiction was given almost 70 years ago in the remarkable study of the Russian physiologist Verigo (1888). Unfortunately, his work remained unnoticed and unrecognized by his contemporaries, probably because of the high authority of Pflüger, whose postulates he attacked. Only recently, thanks to the papers published by Averbakh (1948), Khodorov (1950a-1950d) and Ushakov et al. (1953), the studies by Verigo again drew the attention of physiologists.

According to one of the basic concepts of contemporary physiology formulated by Pflüger (1859), when a constant current is passed through a conducting fiber, the excitability of the latter increases in the region of contact with the cathode, and decreases in the region of the anode. When the current is discontinued the position is instantaneously reversed. The phenomenon is detected by applying the exploring electrodes in the immediate vicinity of the polarizing electrodes.

These phenomena, called "physiological electrotonus" were, and still are, especially important in understanding the formation of a moving wave of excitation at the cathode. According to Pflüger, excitation should appear as a result of increasing excitability of the fiber.

Pflüger was of the opinion that electrotonic changes in excitability arise from far-reaching molecular changes in the protoplasm of nerve and muscle fibers. It was also known that on passage of current, electric potentials of the same sign as those on the electrodes appear in the regions of the nerve surface in the vicinity of the electrodes. Pflüger called these potentials physical electrotonus, and he thought that they were a result of those molecular rearrangements in the protoplasm which cause changes in excitability.

Somewhat later (1883), Verigo observed that increased excitability at the cathode began to decrease following prolonged passage of current. This he called "cathodic depression". The term became firmly established and physiologists, in referring to Verigo's studies, usually regard it as a secondary phenomenon replacing the initial increase in excitability at the cathode.

Verigo's Concept of Pflüger's Electrotonus

Continuing his studies on electrotonus, Verigo (1888) substantially modified his original concept of cathodic depression; he also critically analyzed Pflüger's theories.

356 The reason for Verigo's change of opinion was the work of Hermann (1874, 1886) in which the latter revised the older ideas of DuBois-Reymond and Pflüger on physical electrotonus. This is what Verigo himself wrote on the subject (1888, p. 6): "Since Pflüger's book appeared, Hermann in a series of brilliant studies developed a new theory of galvanic phenomena of electrotonus. This changed our ideas of electrotonic current, and therefore also our concept of the nature of physiological electrotonus. Guided by this theory we should recognize that electrotonic currents are only branches of polarizing current which, thanks to special purely physical conditions, spread into the extrapolar space far beyond the limits of the polarized sector. Thus, the basis of galvanic phenomena of electrotonus is purely physical. This being so, it is natural that the same conditions may also cause the Pflüger phenomena, and that these phenomena depend on branches of polarizing current which may penetrate the whole sector of the nerve in which they are observed".

Indeed, if it is accepted that current enters the nerve fiber not only immediately beneath the electrodes, as Pflüger thought, but also at a certain distance from them, then determination of excitability by the use of testing electrodes must take into account the fact that loops of polarizing current strengthen the force lines of the testing current, and distort the true evaluation of nerve excitability in the vicinity of the electrodes. Obviously in such a case, a current of smaller intensity is sufficient in the vicinity of the cathode to reach the excitability threshold, since it would be superimposed on the loops of the polarizing current. But at the anode these loops will weaken the testing current, since they have an opposite direction. As a result, a false impression is given of increased

excitability at the cathode and its decrease at the anode. It is amazing that such a simple thought did not occur to any physiologist except Verigo!

On the basis of these quite reasonable conclusions, Verigo assumed that the real changes in excitability at the poles of constant current are completely opposite to those suggested by Pflüger. Verigo thought that immediately after the introduction of the current, excitability began to decrease at the cathode and increase at the anode. However, when excitability is determined in the vicinity of the polarizing electrodes, these changes are distorted by the presence of current loops spreading far beyond the limits of the area of direct contact with the electrodes. And only with time, when the excitability threshold determined at the cathode becomes higher than the initial one, does cathodic depression occur.

According to Verigo, the real changes in excitability at the poles are observed only on rapid discontinuation of the current; in this case, excitability at the cathode decreases while that at the anode increases.

Verigo arrived at these simple conclusions while studying excitability during interrupted current. He found that the effect of such a current depended not only on the duration of the impulses, but also on the duration of the intervals between them. It was found that only when the intervals were sufficiently long was tetanic muscle contraction obtained in response to any 357 stimulation. However, when the intervals were too short, only a single muscle contraction occurred at the first instant of stimulation. The effect was the same as on stimulation by constant current. Verigo's explanation was based on the assumption that all excitations, even those caused by short current impulses, cause a decrease in excitability at the cathode, which gradually returned to normal on discontinuation of the current.

At present, this assumption no longer seems paradoxical, since it is known that each excitation is accompanied by refractivity, i.e., by decrease in excitability. If the interval between the impulses was so short that lowered excitability (refractivity) did not have time to return to its initial values, and the new current pulse was subthreshold in intensity, then only the first impulse could cause a stimulus and a single contraction of the muscle. All subsequent electrical stimulations were subthreshold and did not cause a response reaction in the muscle. However, if the intervals between stimulations were prolonged to such an extent that the lowered nerve excitability had time to return to its initial level, the nerve responded to each stimulation, and the muscle developed tetanic contraction.

These experiments led Verigo to the conclusion that cathodic depression, or the decreased excitability at the cathode* appears immediately after closing the circuit of the constant current. However, the phenomenon is barely seen when excitability is investigated by placing the testing electrodes in the vicinity of the polarizing ones, because the current loops spread to a considerable distance from the latter.

Verigo illustrated his ideas by a diagram, as illustrated in Figure 205. Here the line oo' illustrates the case of a nerve to which the cathode of polarizing current is applied at point b. The anode is located somewhere to the right outside the drawing. The curve ab'c'd'e'f'g' shows the distribution of catelectrotonus at various points of the nerve, in both directions from the site of application of the cathode (d). The current leaves the nerve not only immediately at the site of contact with the electrode,

---

* In contemporary terminology—refractivity of the arising excitation.

but also at some distance from the latter, the intensity of the outgoing current diminishing with increase in the distance from the electrode (dd', cc', bb').

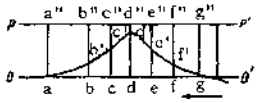


FIGURE 205. Diagram illustrating the relationship be-
358 tween polarizing and testing currents in the study of electrotonus (according to Verigo, 1888)

oo'—nerve; d—site of application of the cathode of polarizing current; ab'c'd'e'f'g'—curve of distribution of catelectrotonus (polarization); the distance from oo' to pp' (aa'', gg'') is the threshold of the nerve before polarization; b'b'', c'c'', d'd'', e'e'', f'f''—are the values of the testing current in order to reach the threshold (apparent excitability thresholds).

Figure 205 illustrates a case in which the polarizing current is below threshold and therefore cannot cause an excitation wave. Let us imagine that immediately after closing the circuit of the polarizing current we start to determine excitability by the ordinary method, at various distances from the electrode. If this check will be performed at a sufficient distance from the electrode, at point a the threshold value will be determined by the segment aa'' which equals the normal threshold of the nerve. However, if we determine the threshold closer to the electrode at point b, a weaker current equal to b'b'' will be required to reach the threshold current intensity, since this current will be augmented by the loop of polarizing current present in this area, equal in intensity to the segment bb'.

In classical experiments on electrotonus this loop is not taken into account, and therefore the decreased threshold is considered by the investigator to be a result of increased excitability at the cathode. That is the result obtained immediately after closing the circuit of the polarizing current. According to the data of Verigo, progressive decrease in excitability at the cathode immediately begins. As a result, the excitability of the nerve (bb'') begins to increase and therefore the addition (b'b'') to the current loop (bb') which is usually accepted as the true threshold of the nerve in electrotonus, will also begin to increase. When this value exceeds the initial threshold of the nerve (aa''), cathodic depression will set in.

Accordingly, Verigo also dissussed the phenomena taking place at the anode of direct current, explaining them in a similar way. In his opinion, the excitability of the nerve actually increases at the anode, but initially the wrong impression is created that a decrease in excitability occurs because the loops of the polarizing current lower the intensity of the testing current.

In the light of Verigo's views, the alteration of electrotonic changes at the break of the current which was described by Pflüger receives a well-founded explanation. According to Pflüger, this phenomenon consists of: (1) the instantaneous decrease (at the cathode) or (2) increase (at the anode) of the excitability of the nerve which had been increased following the break of the current. After the lapse of a certain period of time, the excitability returns to its initial level. The instantaneous nature of the mentioned reversible transformations was most puzzling. Such an incredible rapidity of this process was stranger still because the subsequent restoration of excitability was comparatively slow. In the opinion of Verigo, a simple solution to this problem is that, at the break of the current, no instantaneous

transformations in the protoplasm of the nerve tissue occurs, but merely an instantaneous disappearance of current loops takes place. This creates a false impression of increased excitability at the cathode, while in reality an increasing depression took place.

By breaking the current we at once removed the current loops, i.e., the source of the errors which distorted the true picture. We immediately observed the excitability changes which really take place in the nerve under the influence of constant current, namely, decreased excitability at the cathode and increased excitability at the anode. The latter does not take place instantaneously, but after a known, measurable time interval, during which metabolic processes essential for repair of the decreased excitability have had ample time to terminate.

The Theory of Verigo and So-called Accommodation

Further on in his remarkable study, Verigo showed how it is possible, by application of his theory, to explain why the excitability threshold of
359 a nerve is higher in the case of a slowly increasing current than with a rapid increase. DuBois-Reymond explained this by the fact that the onset of nerve excitation is not dependent on the intensity of the current but on its fluctuations.

According to Verigo, slowly increasing current cannot cause excitation, because immediately after closing the current circuit, the excitability threshold of the nerve at the cathode begins to increase, this increase being able to take place more rapidly than the increase in current intensity. In that case, if the rate of increase of the current exceeds the rate of the decrease in cathodic excitability, excitation should arise and its threshold should be higher, if the steepness of increase of the stimulating current is smaller. This concept is illustrated in Figure 206. Time is plotted on the abscissa, and intensity of stimulating current on the ordinate. The line OBB^1 illustrates the development of instantaneously increasing current. Under such conditions, the segment OB corresponds to excitability threshold. However, according to Verigo's data, from the first moment of closing the current circuit the excitability threshold begins to increase steeply along the line BkD (increase in cathodic depression). The diagram shows that only in the first moments will the current OBB' have above-threshold value. From the moment k, the current becomes subthreshold, and can therefore not cause excitation in the nerve. For this reason, excitation appears only at the instant of closing the constant current circuit.

The line OE (Figure 206) illustrates a current which increases gradually and in linear fashion. Here, as in the previous case, the threshold begins to increase from the first moment of closing the current circuit (increase in cathodic depression). But due to the fact that the current increases gradually (OE), and the rise in threshold (BF) is slower than in the case of rapid closure of the current circuit (BD), no excitation arises because the increasing current cannot reach the level of excitability threshold of the nerve which increases the distance between them. In the case of a steeper increase in the current, the latter may reach threshold value. In this case, excitation arises, but its threshold is higher than in the case of instantaneously increasing current.

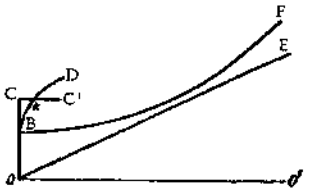


FIGURE 206. Diagram illustrating the role of the steepness factor in increase of current causing excitation (according to Verigo, 1888)

OBkD—increase in excitability threshold of the nerve in the case of instantaneously increasing current OCC'; BF—increase in excitability threshold of the nerve in case of linear increase of current OE; OB—threshold excitation of normal nerve.

On this basis Verigo's conclusions were contrary to the generally accepted ideas of DuBois-Reymond, according to which constant electric current does not in itself cause excitation. Excitation supposedly appears due to changes in current intensity, while according to Verigo, "galvanic current in the sphere of its catelectrotonus possesses a constant excitatory capacity of the same value at the moment of closing the circuit, as at the time of passage in the nerve with unchanged intensity". And if there is no excitation, or when the threshold of excitation increases upon "creeping in" of the current, this may be explained by "the capacity of catelectrotonus to lower excitability, starting from the first moments of polarization".

Thus, according to Verigo, from the very onset of nerve excitation there is a decrease in its excitability. How can this phenomenon be defined?

It is known that when an excitation wave passes along the nerve fiber, the excitability of the latter at first decreases and later disappears entirely. This phenomenon was named "refractivity of the excited segment". In the previous chapters of this book we repeatedly indicated that refractivity accompanies excitation states of all types—propagating as well as local. This is entirely
360 logical, since no tissue can be infinitely excited, and each excitation has its own limits. Consequently, if a tissue is already excited, its excitability should decrease, i.e., refractivity should set in, a point which was previously discussed in detail. It is also known that electric current acts as an irritant in propagating excitation. It follows from this that refractivity which appears in the case of an excitation wave, and cathodic depression observed by Verigo after the action of constant electric current on the nerve, are similar phenomena.

The ideas developed by Verigo in his book published in 1888 may be considered as a general theory regarding the basic phenomena of nerve excitability. This theory was in sharp conflict with the ideas of the greatest authorities in physiology at that time, namely, Pflüger and DuBois-Reymond.

This probably explains why Verigo's work remained entirely unnoticed and unappreciated by his contemporaries. Only now are physiologists beginning to arrive at those conclusions reached by him 70 years ago.

The first to point this out was Averbakh (1948), who has shown that Verigo's explanation of the phenomenon of "creeping in" of gradually increasing electric current is in fact an anticipation of Hill's famous theory of accommodation (1935, 1936). Figure 207 illustrates Hill's explanations of this phenomena. Line V designates increase in potential arising at the surface of the nerve on passage of current, line U being the increase in excitation threshold of the same nerve. U_0 is the threshold in the case of instantaneously arising current. If the current increases gradually until

it reaches the threshold, the latter has time to increase in comparison with U_0 (Figure 207, D). The less steep the increase in current is, the higher the threshold of excitation (Figure 207, C). Finally, at a certain limit slope of current increase, curves V and U will not intersect at all, and consequently no excitation will ensue (Figure 207, B, A).

361

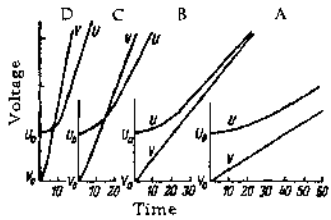


FIGURE 207. Relationship between steepness of current increase (V) and change in excitability threshold of the nerve (U) (according to Hill, 1936).
For explanation see text.

As seen from the above, both Hill (1935) and Verigo (1888) started from the assumptions that (1) excitability threshold of the nerve starts to increase at the cathode as soon as a constant current is passed, and (2) that the increase in current and threshold do not take place according to the same law, which may result in intersection of these two curves. It is amazing that even the diagrams given by the two authors are very similar (compare Figures 206 and 207, B, A). However, Hill did not refer to the work of Verigo carried out almost half a century earlier.

There are, however, differences between the viewpoints of the two authors. The most important refer to the interrelationships between so-called accommodation and Pflüger's electrotonus. We have already said that Hill was not able to explain the contradiction between "the accommodation increase in threshold at the cathode" and "electrotonic decrease in threshold at the cathode" with passage of current. In order to extricate himself from this situation, he assumed that these are two different phenomena not occurring simultaneously. It will be seen later that this assumption was wrong. As far as Verigo is concerned, this contradiction was nonexistent, since according to his theory, excitability does not increase at the cathode attached to a nerve, as Pflüger thought, but decreases. According to Verigo, "cathodic depression", "accommodation", "catelectrotonus" and "refractivity" are all similar phenomena.

In this respect the term "accommodation" may be elaborated. We have already pointed out that (p. 327) this term was introduced by Nernst to explain the independence of excitability threshold and the time of action of adequate current impulses. It was assumed that on prolonged action of the current the nerve becomes somehow adapted (accommodated) to the stimulus, and therefore a large dose of current is necessary in order to produce a response reaction. It is self-evident that "cathodic depression" of Verigo, or the "refractory phase" have nothing whatsoever to do with any adaptation.

However, many physiologists, and especially members of the medical profession, understand accommodation literally, as a manifestation of a special property of the nerve to adapt itself to surrounding conditions. This partially explains the great popularity gained by this method for diagnostic purposes.

A number of authors (Erlanger and Blair, 1931; Blair and Erlanger, 1936a; Granit and Scoglund, 1943; Scoglund, 1945) also indirectly concluded that similar phenomena form the basis of accommodation and cathodic depression. However, none of these authors formulated this idea as lucidly and as completely as Verigo.

Studies Confirming Verigo's Theory

Verigo's theory has recently been checked and fully confirmed by Khodorov (1949, 1950a-1950e, 1951) on the basis of more refined modern physiological methods.

Verigo reached his conclusions which contradict the data of Pflüger on changes in nerve excitability at the poles of a constant current; this was mainly based on studies of the action of interrupted current on the nerve. Therefore, Verigo's proofs were to some extent indirect. Khodorov aimed at studying the change of nerve excitability directly, in the area of attach-
362 ment of the electrodes, and thus confirmed directly the theory of Verigo. For this purpose he used a modified Verigo apparatus, making it possible to polarize and stimulate the nerve by the same electrodes by using current of the same form (Figure 208).

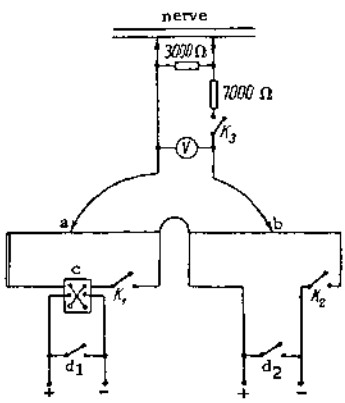


FIGURE 208. Scheme of apparatus for simultaneous polarization and stimulation of the nerve (according to Khodorov, 1950e)
For explanation see text.

The apparatus consisted of two circuits: polarizing circuit (left) and stimulating circuit (right), connected with each other in series by the method of Verigo (1888). The current intensity in each circuit was regulated by moving the indicator of the potentiometer (a or b). Since the resistance of the rheochord string was weak, the current intensity of one circuit was not affected by moving the indicator of the other. The direction of the current in the polarizing circuit was regulated by a 6-way switch (c). The direction of the testing current was always downward. Closing the circuit of the testing and polarizing currents was performed by turning on the switches (k_1 and k_2) by hand (if excitability was determined with protracted intervals of polarization), or by breaking switches of the Helmholtz pendulum (d_1 and d_2), if the thresholds were measured with short intervals of polarization (from 10 to 60 msec).

In order to counteract the effect of changes in resistance of the nerve during polarization, Lapicque's shunt was included in the apparatus. The nerve was stimulated by nonpolarizing electrodes.

By this means changes in excitability of the nerve under the influence of constant current could be investigated directly under the cathode. One such experiment is illustrated in Figure 209. At first the excitability threshold was determined with instantaneously increasing current (V_o, rheobase). Later, constant (polarizing) current of subthreshold intensity (V_n) was introduced, and the additional voltage (ΔV) necessary to reach the threshold value at various time intervals after connecting the polarizing current was determined. It is self-evident that at the moment of switching-on the current, the addition ΔV was equal to the difference between V_o and V_n ($\Delta V + V_n = V_o$). Later, in the first $1\frac{1}{2}$ msec, according to Khodorov, the value of the addition ΔV remained the same. Therefore the excitability threshold or the excitability of the nerve did not change. However, after 2 msec, as seen from Figure 209, in order to obtain excitation it was not

ΔV that had to be added to the polarizing current (V_n) but a higher value, ΔV_1. Therefore the threshold excitation increased while nerve excitability decreased. Figure 209 shows that with each msec the threshold value increased, this increase being faster initially, slowing down later.

363

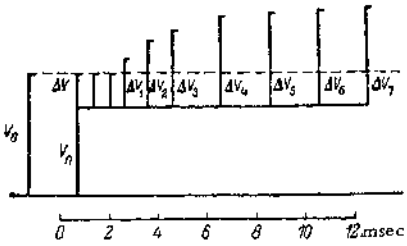


FIGURE 209. Change of cathode excitability thresholds during the first msec of polarization of the nerve (according to Khodorov, 1950e)

V_o—threshold the case of instantaneous closing of the circuit; V_n—value of polarizing current; ΔV, ΔV_1, ΔV_2.... ΔV_7—additions of testing and polarizing current necessary to induce excitation after various time intervals.

From these easily reproducible experiments, it follows beyond any doubt that Verigo was correct in assuming that decreased excitability at the cathode (cathodic depression) begins from the first msec and that no Pflüger increase in excitability at the cathode takes place. In terms of Pflüger's theory excitability was measured not by the value of the total current passed through the nerve ($V_n + \Delta V$), but by the value of the addition to the polarizing current (ΔV), as he himself did. As seen from Figure 209, this addition (ΔV) is indeed smaller than the initial threshold (V_o). From this it may be concluded that excitability increased at the cathode. However, the error of this assumption in the experiment suggested by Khodorov is quite obvious.

That excitability only decreases at the cathode from the first msec can be seen in experiments where the polarizing current was broken rapidly. One such experiment carried out by Khodorov is illustrated in Figure 210. After closing the circuit, the thresholds begin to increase, reaching a value of $V_n + V_{10}$ after 8 msec. Immediately after breaking the polarizing current the excitability threshold (V) remains initially as before ($V = \Delta V_{10} + V_n$) and later begins to drop, returning to the initial value (V_o) within several msec. This is the true picture of altered cathodic excitability on making and breaking the constant current circuit.

Pflüger's viewpoint is somewhat different. After closing the circuit of constant current (V_n), the magnitude of the latter is no longer included in the evaluation of excitability, and the threshold is determined only from the testing voltage ΔV, which must be added to V_n in order to reach the threshold. Since the value of ΔV is smaller than the initial threshold V_o, a state of increased excitability exists. Immediately after breaking the polarizing current, a voltage V is necessary in order to obtain excitation, and since V is larger than ΔV_{10} the increase in excitability is instantaneously transformed into lowered excitability, later slowly returning to the initial value.

364 The error of this opinion is obvious. Besides, it is quite improbable that molecular rearrangement from increased to decreased excitability occurs in the nerve immediately after breaking the current. It is also difficult to understand why the threshold of this new lowered excitability (V) is always equal initially to the sum of the value of the previous threshold (ΔV_{10}) and the value of the polarizing current (V_n). As can be easily seen

from Figure 210, in reality no instantaneous shift in excitability takes place. The threshold increases upon passage of current ($V = \Delta V_{10} + V_n$), and gradually returns within several msec to the initial value.

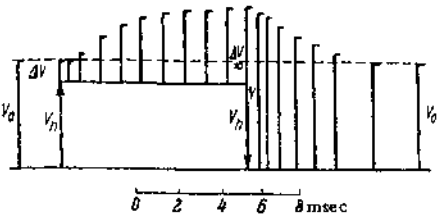


FIGURE 210. Change of cathode excitability thresholds of the nerve on making (↑) and breaking (↓) the circuit (according to Khodorov, 1950e)

V—threshold voltage after breaking the polarizing current. Remaining legend as in Figure 209.

Figure 211 shows the effect of temperature on the speed of this return to the initial value. After 10 msec, at a temperature of 2° C, the threshold reaches only 150% of the initial value, at 18° C—125% and at 29° C (within the same time period)—the threshold drops to such an extent that it remains 10% below the initial value. This distinct dependence on temperature indicates that the processes of repair, returning the excitability threshold of the nerve which increased at the cathode to its initial value, are first of all determined by the speed of metabolic reactions.

Later the change in nerve excitability at the anode of constant current was investigated, using the method of Khodorov. The results of one such experiment are illustrated in Figure 212. At first the initial threshold of the nerve—V_o (rheobase) was determined. The voltage of the polarizing current (V_n) was plotted on the graph below the central line, since its sign was opposite to that of the voltage of the testing current. After closing the circuit, the excitability threshold of the polarized nerve was determined at various moments after zero time. Immediately after closing the circuit a voltage of ΔV was necessary to reach the threshold. However, in view of the fact that a voltage of an opposite sign equal to V_n was already applied to the nerve, the actual voltage
365 was $\Delta V - V_n$. As seen from the figure, this value equals the initial threshold of the nerve V_o Consequently, immediately after closing the circuit there was no change in nerve excitability.

Usually the value of the polarizing current is not taken into account, and excitability is determined by the total value of the added voltage ΔV, not subtracting from it the voltage V_n. In that case the conclusion is that immediately after closing the current, circuit excitability decreases at the anode, since $\Delta V > V_o$. According to Khodorov, the threshold of the nerve at the anode does not change for the first 2 msec ($\Delta V = \Delta V_1$), beginning to decrease later ($\Delta V_1 > \Delta V_2 > \Delta V_3 > \Delta V_4 \ldots$). In other words, excitability at the anode, starting from the second msec, increases progressively, contrary to Pflüger's law.

Figure 213 shows the results of an experiment in which we investigated the change of nerve excitability at the anode after breaking the circuit of constant current. After closing the current circuit the true thresholds of excitability of the nerve decreased $(\Delta V_1 - V_n) > (\Delta V_2 - V_n) > (\Delta V_3 - V_n) \ldots$ Immediately after breaking the current circuit, the values of these thresholds remained the same, but further on, they began to increase, reaching the initial value after several msec. These data contradict all previously known facts. According to Pflüger's theory of electrotonus, on closing the circuit of the current, the excitability thresholds of the nerve at the anode increase,

while on breaking the circuit the lowered nerve excitability immediately changes to an increased excitability. The source of error of this statement is the same as in the case of the cathodic changes of excitability, and is due to the fact that the investigators did not take into account the voltage of the polarizing current which must be subtracted from the total voltage of the testing current. If this is done, no reversion of excitability on breaking the current circuit is observed.

The studies of Khodorov were repeated in our laboratory by Ushakov, Averbakh, Suzdal'skaya, Troshin and Cherepanova (1953) and were basically confirmed.

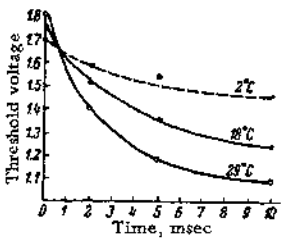


FIGURE 211. Effect of temperature on speed of restoration of the threshold of nerve excitability at the cathode, after breaking the polarizing current ("post cathodic depression") (according to Khodorov, 1950e) The voltage of the polarizing current was 90% of the rheobase value. The duration of polarization was 1 sec. Time after breaking the current is shown on the abscissa. The values of the thresholds, expressed as multiples of the rheobase, are shown on the ordinate.

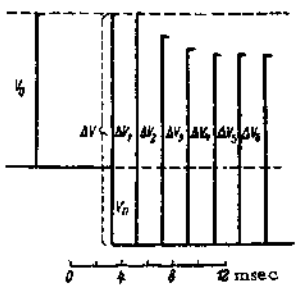


FIGURE 212. Changes in excitability thresholds at the anode during polarization of the nerve (according to Khodorov, 1950e) V_n = 50% rheobase. ΔV, ΔV_1, ΔV_2, ΔV_3, ΔV_4, ΔV_5, ΔV_6—thresholds measured directly: $\Delta V - V_n$, $\Delta V_1 - V_n$, $\Delta V_2 - V_n$, $\Delta V_3 - V_n$, $\Delta V_4 - V_n$, $\Delta V_5 - V_n$, $\Delta V_6 - V_n$—are the true excitability thresholds. The remaining legend as in Figure 209.

366 Figure 214 shows the curve of increase in excitability thresholds at the cathode, as obtained by us, very similar to that obtained by Khodorov. It differs only in that it shows no latent period within the first 2 msec (not observed by the authors), but described by Khodorov. In experiments carried out by these authors, the thresholds began to increase immediately after closing the current circuit. The same was true at the anode (Figure 215). These results were very similar to those obtained by Khodorov, but here, too, there was no latent period. This is the single detail of Khodorov's

work which Ushakov and co-workers were not able to confirm. In addition, these authors observed that when weak polarizing current, not exceeding 5-10% of the rheobase, was applied to frogs kept in the cold, the nerve excitability at the cathode might increase somewhat during the first msec (about 10%). When a stronger polarizing current was used (60%) this increase was entirely absent.

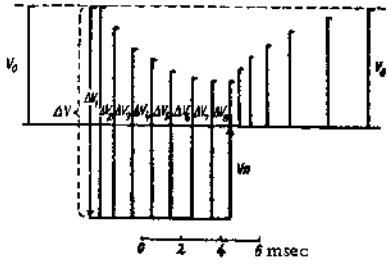


FIGURE 213. Change in excitability thresholds at the anode on closing (↓) the circuit, and on breaking (↑) it (according to Khodorov, 1950e). Legend as in Figures 209 and 212.

Khodorov has shown by a number of convincing experiments (1950b-1950d) that Hill's assumptions were wrong. He determined the curve of the true catelectrotonic changes in the excitability thresholds, and the curve of speed of accommodation for the same nerve almost simultaneously, and through the same electrodes by the generally accepted method. He compared these curves with each other. Figure 216 shows the changes of both these values with the temperature fluctuating between 17 and 28° C. As can be seen, the two curves are detailed reproductions of each other. Figure 217 shows the comparison between the changes of these two indexes of the nerve under different circumstances*. Here, also, the two curves bear a striking re-
367 semblance to each other. This is seen not only from the fact that they decrease simultaneously at the beginning and then rise, reflecting the biphasicity of the effect of the excitants, but also from the fact that they both repeat the small changes evidently due to chance (for example, a small plateau in the upper right part of the curve in Figure 217, A). There is no doubt that the two curves reflect changes of the same property of the nerve fiber.

The new data given by Khodorov and by Ushakov and co-authors confirming Verigo's hypotheses are so convincing that they cannot be ignored. These data have so far found no recognition in scientific literature. The point in question is a revision of the basic laws of electrophysiology as
368 stated by Pflüger, laws on the basis of which various wide theoretical generalizations were made. The revision of Pflüger's laws will necessarily lead to the revision of a number of associated concepts.

Table 72 illustrates the classical hypotheses which should be reviewed on the basis of Verigo's theory, confirmed by Khodorov, Ushakov et al.

---

* The data of Khodorov on the effect of $CaCl_2$ on the nerve differ somewhat from those of Hill (1935, 1936) and Solandt (1936a, 1936b). The English authors described only an increase of $1/\lambda$, while according to Khodorov's data this stage was preceded by a decrease of $1/\lambda$. A sharp increase of this value, without prior decrease, was observed by Khodorov only in frogs kept at a low temperature.

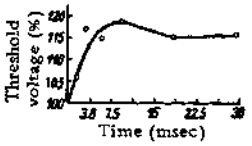


FIGURE 214. Change in excitability threshold of the nerve at the cathode following the action of polarizing current corresponding to 60% of the rheobase. The initial rheobase is taken as 100% (according to Ushakov and others, 1953)

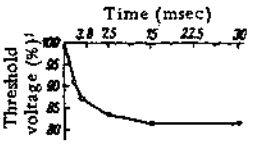


FIGURE 215. Change in excitability threshold of the nerve at the anode following the action of a polarizing current corresponding to 60% of the rheobase. The initial rheobase is taken as 100% (according to Ushakov and others, 1953)

FIGURE 216. Change in speed of accommodation (1/λ), and speed of development of cathodic depression (CET), after heating and cooling of a nerve (according to Khodorov, 1950e).
The temperature corresponding to each measurement is given on the abscissa; the speed of accommodation CET is given on the ordinate.

369

Origin of Spreading Excitation

It has been mentioned above that the great interest always shown by physiologists in the theory of electrotonus was due to the fact that this theory dealt not only with changes in nerve excitability at the poles of constant electric current, but also gave a satisfactory explanation, at first sight, of the appearance of excitation at the cathode. It was assumed that excitation arises as a result of increased excitability of the fiber. However, according to Verigo, there is no increase in excitability at the cathode. On the contrary, the cathode is characterized by decreased excitability. We fully agree with this concept.

Why then does a wave of excitation originate, after all, at the cathode?

The answer to this question follows from all that was said in the chapter on spreading excitation. It was indicated there that we accepted the theory of "small currents" of Hermann as a basis of the concept of spreading excitation. According to this theory, an excitation moving without decrement should appear when at each point of the conducting fiber a sufficiently sharp border has formed between the excited electronegative surface of the fiber and the adjacent nonexcited, positively charged segment.

Table 72

Comparison of Pflüger's basic concepts of electrotonus, and those arising from Verigo's theory

Hypotheses accepted in contemporary physiology	Changes introduced by Verigo
There is an initial increase in nerve excitability at the cathode of constant current.	Nerve excitability decreases immediately at the cathode of constant current*.
Nerve excitability decreases at the anode of constant current.	Nerve excitability increases at the anode of constant current.
On breaking a constant current, the increased nerve excitability at the cathode is instantaneously transformed into lowered excitability. The lowered excitability at the anode is transformed into increased excitability. Following this the changed excitabilities gradually return to normal.	On breaking a constant current, the increased excitability at the anode and the decreased excitability at the cathode initially remain unchanged. Later the excitabilities at both poles gradually return to normal.
Increase in excitability at the cathode, refractivity, and accommodation, are different phenomena.	Decrease in excitability at the cathode, refractivity, and accommodation, are similar phenomena.

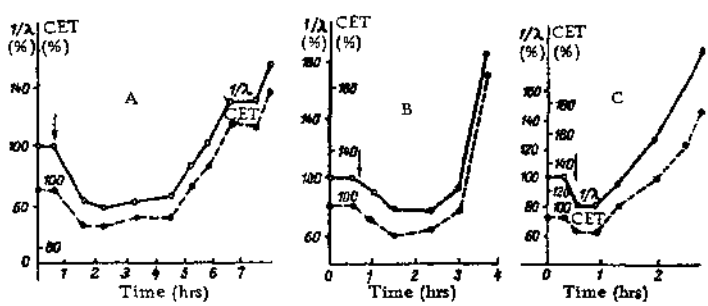


FIGURE 217. Comparison of speed of accommodation (1/λ), and speed of cathodic depression (CET), in the process of alteration of the nerve (according to Khodorov, 1950e)
A—1.24% M solution of $CaCl_2$; B—0.5% M monoiodoacetate; C—1/500 M solution of cyanide. Onset of alteration is designated by an arrow. Values of 1/λ and CET before the beginning of nerve alteration are taken as 100%.

---

* A small and brief increase may be observed only with very weak polarizing currents in the cold.

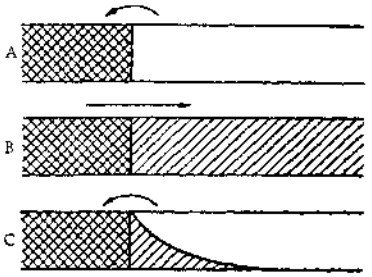


FIGURE 218. Diagram illustrating the lack of a multiple response to a single stimulation. For explanation see text.

Consequently, one of the conditions for appearance of a single spreading impulse is a sufficiently strong, local, separated reaction from an irritant (mechanical, electrical, chemical, etc.). It can be shown that as a rule only one impulse may emerge from such a sharply delineated excited zone. Indeed, let us assume that such an electronegative zone (Figure 218, A) was created by the action of a certain constant stimulus. (cathode of direct current, pressure, etc.). Let us assume that the difference between the potentials of the altered (shaded on the drawing) and intact zones is so great, that conditions were created for nondecremental conduction, and that the whole fiber was engulfed by an excitation wave (Figure 218, B). Later, repair of the excited fiber begins, but conditions for restoration are not uniform. Away from the site of action of the constant stimulus these conditions are favorable, and restoration will reach completion. As far as the region adjacent to the site of the constant stimulus (Figure 218, C) is concerned, restoration will invariably be made difficult by the resultant current of action. The effect of this current will be greater, the closer it is to the border of the alteration. As a result, the previous sharp border between the altered and restored part of the fiber will no longer be existent. There will always be a gradual transition between the area of constant action of the stimulus, and the nonstimulated part (Figure 218, C), due to which a secondary impulse will not arise.

The foregoing concerns the formation of single waves of stimulation. As has been shown in Part IV of this book, another cause of impulses may be increased excitability. However, in contrast to the previous case, it is
370 rhythmic activity rather than a single impulse which occurs after a sufficient increase in excitability.

In the organism under natural conditions a series of consecutive excitation waves rather than single impulses originate in the centers and nerve endings, generally speaking. Thus, the main method of transporting impulses along nerve fibers should be considered to be a more or less prolonged rhythmic activity and not single impulses. Consequently, it may be assumed that the main mechanism of propagating impulses in the nervous system is a local increase in excitability, either somewhere in the nerve centers or in the nerve endings.

Very little is yet known regarding the factors which may raise excitability of nerve centers during normal function of the nervous system. As far as the nerve endings are concerned, some speculation is possible. It has already been mentioned that the reason for appearance of rhythmic impulses here may be the initial stage of action of the stimulus (described by Vvedenskii), leading to increased excitability. Needless to say, this requires further experimental confirmation.

371 CONCLUSIONS

At the beginning of this book we defined the term "cellular excitation" (p. 40)

This term denoted reversible changes in protoplasm due to changing external conditions. This stimulated various useful activities of the cell. Essentially the changes involved reversible alteration of cell proteins, similar in nature to denaturation.

By comparison with former definitions, the current approach is an attempt to introduce into the definition specific concepts of physicochemical changes in the living substrate, in addition to the ordinary functional attributes of cellular excitation. The proposed definition is therefore more exact, and integrates a group of phenomena related both in physicochemical nature, and in their phylogenetic origin.

The latter statement was supported by evidence that cellular excitation influenced the capacity of protoplasm to repair damage in the substrate, caused by changes in the environment. Without this property even the most primitive unicellular organism could not exist.

In this form of excitation the damage factor is also a stimulus, and the response reaction should be considered as a chain of transformations directed mainly towards the repair of the defect. Such reversible changes in protoplasm and the cell nucleus may be observed in any tissue at the site of application of the stimulus. These changes were denoted as local excitation (in contrast to excitation propagating (spreading) along a conducting fiber). The reversible changes in protoplasm (colloidal, sorptional and other changes) were studied in detail during local excitation, at the site of the stimulus. These changes were called paranecrosis.

The next stage in evolution of the excitation process should be considered as the capacity of the cell not only to repair the damage caused by the stimulus, but also to avoid it or protect itself against it. Other activities useful for the unicellular organism are associated with this capacity.

With the appearance of multicellular organisms and the evolution of the nervous system, a higher form of cell excitation emerged, i.e., excitation which propagated along conducting fibers.

This form of excitation was also shown to be based on reversible
372 denaturation of protoplasmic proteins. There are reasons to believe that as a result of denaturation, certain substances are released which were bound to the proteins in the resting condition (for example, potassium, phosphates, creatine). It is highly probable that the mechanism of excitation initiating these or other biochemical processes is based on this release of substances.

The protein theory of excitation developed in this book, according to which alterations in the proteins of the entire mass of protoplasm form the

basis of excitation, is in contradiction to the membrane theory prevailing in contemporary physiology. This states that the appearance of cellular excitation and of bioelectric potentials are due to increased permeability of the protoplasmic surface membrane.

Investigation of cellular permeability led us to reject the theory of the so-called semipermeable boundary membranes controlling the entrance of different substances in the cell, the appearance of excitation, and bioelectric phenomena.

We concluded that the majority of phenomena associated with cellular permeability can be explained by the concept of protoplasm as a complex coacervate system, the water of which is a poor solvent for the majority of substances and behaves like a nonaqueous phase in relation to the surrounding aqueous solution. The intracellular content of certain substances is higher than that of the surrounding medium since they are adsorbed onto micellular surfaces and are chemically bound. The reason for bioelectric phenomena is the transition of a part of the protoplasm electrolytes from the bound to the free state.

In the present book we have laid foundations for the concept that there is no fundamental difference between local excitation of the cell arising in the area of application of any stimulus, and excitation spreading along a conducting fiber. This idea was first expressed by Vvedenskii. It was the basis of his theory of parabiosis. The main arguments in its favor are the following:

1. The similarity between physicochemical changes in protoplasm in the case of local and spreading excitation (increased staining power, decreased dispersion of colloids, increased viscosity, etc.).

2. The similarity between biochemical reactions occurring during local and spreading excitation.

3. The development of, first relative, and later absolute refractivity (decreased electric excitability in the stimulated area), in local and in spreading excitation.

4. The tetanic contractions occurring in skeletal muscles, in the case of both local and spreading excitation.

5. Electronegativity accompanying both local and spreading excitation. In local excitation stable electronegativity is observed. According to the generally accepted theory of electric propagation of an impulse, the reason for excitation of each point of the conducting fiber is the electric current generated by the adjacent stimulated segment. Consequently, in
373 the case of a spreading impulse, excitation of each segment of the fiber may be considered as a local effect caused by the cathodic current.

Objections have often been raised against classifying local reactions in conducting fibers, together with spreading impulses, on the grounds that the latter are basically different from the former, since they behave according to the so-called "all or none" law, while local reactions do not obey this law, and gradual relationships between the magnitude of excitation and that of the response effect are characteristically seen in their case.

Special attention is devoted to this problem in the present book. Detailed analysis of relationships between intensity of electric excitation and magnitude of electric response reaction led us to the formation of the theory of gradual excitation. According to this theory, there is no difference between the local reaction spreading with decrement, and the impulse moving without decrement. Those phenomena, which in the opinion of the majority

of physiologists obey the "all or none" law, are not related to the onset of an excitatory state, but with the characteristics of conduction of excitation.

The theory of gradual excitation enabled us to explain and elucidate certain phenomena still unknown to physiologists. These are associated with the appearance and spread of excitation, and the experimental proof of their existence. As an example of such a confirmation we cite the possibility of obtaining local potentials considerably greater in magnitude than those of the propagating peak, which spread with decrement until they reach the dimensions of the peak potentials (above-peak potentials).

The use of quantitative methods of investigating the changes in protoplasm after excitation compels us to discard the concept of the excited and resting conditions of the cells as two alternative terms. Instead, we acknowledge the existence of a continuous series of transition stages in protoplasm. These start from a level characterized by a high degree of normality of the proteins, high excitability and a more positive electric potential, and end with increasing disorders in these factors and in a state approaching death.

From the above it follows that the process of excitation of the cell consists of a transition of protoplasmic proteins from a higher level of activity to a lower one under the influence of the stimuli, and that this transition is in all probability a stimulus for a chain of biochemical transformations essential for various cellular activities and for the return of the protoplasm to its initial state.

374 If this is so, then excitation of the cell should be considered not as a state but rather as a cyclic process, due to which the protoplasm passes through a series of phases. Such a consecutive change of phases is observed during passage of a nerve impulse along a fiber, whereby the sum of changes as a whole was often called by us "a wave of excitation", thus emphasizing the cyclic nature of the entire process. The same is true for the development of the more primitive local excitation. Here, too, a cycle of events takes place, but does not terminate after a few thousandths of fractions of a second, as in the case of an impulse propagating along a fiber, but may continue for minutes or even hours.

Physiologists who have investigated various aspects of the nervous system have often used the term "blocking" as opposed to the term "excitation". In the physiology of the nervous system, as is well-known, the term "blocking" designates clearly defined and highly important phenomena. However, in terms of the elementary processes taking place in the cell, such a concept of two supposedly opposite states of the protoplasm is hardly justified. In the study of intracellular processes, we deal primarily with two phenomena, i.e., the level of protoplasm excitability and the process of excitation proper. The former may be higher or lower. Low excitability creates less favorable conditions for the conduction of excitation and may lead to a block in conduction. Increase in excitability, on the other hand, creates better conditions for conduction and may lead to rhythmic activity. Low excitability of the conducting fiber may certainly be defined as a

"blocking" factor, but this condition should then be contrasted with high excitability, although not to the process of excitation itself*.

In our opinion, in the present state of knowledge of the physiology of the cell, the use of the term "blocking" as a state opposed to the term "excitation" is not rational. We think it more advisable to describe the level of excitability of protoplasm on the one hand, and the process of excitation which develops against a background of this level of excitability, on the other.

---

* In relation to the nervous system of the organism as a whole, physiologists often understand the term "excitation" as a state of increased excitability. By "blocking" they refer to processes causing a decrease in excitability. Such a concept of excitation in the nervous system as a whole does not tally with our definition of cellular elements. Other physiologists use the term "blocking" to mean conditions which according to us are one or other phase of the process of excitation. The question is therefore one of terminology. Blocking cannot be opposed to the process of excitation, since it corresponds only to some phases of this process.

CLOSING NOTES

375 The monograph "Local Reaction and Spreading Excitation" was completed by Dmitrii Nikolaevich Nasonov at the end of 1956.

The central problem which Nasonov studied during the last years of his life was that of the unity of local and spreading excitation. This resulted in the theory of gradual excitation. After the monograph was written, studies were carried out in his laboratory at his initiative, the results of which Nasonov thought extremely important for further experimental confirmation and substantiation of his theory. He proposed to include them in this book. Since Nasonov did not manage to do so, we think it necessary to present these studies in a brief form.

The S-shaped dependence between excitation and the local electric response reaction postulated by the theory of gradual excitation was first observed by Mozhaeva (1958a-1958c) in the sciatic nerve of the frog. Analysis of different segments of the curve expressing this dependence has shown that the S-shaped form obtained cannot be explained by statistical laws of summation of activity of the different fibers. The study of dependence of the magnitude of the local electric reaction on the intensity of excitation, within a large range of intensities, has shown that at high intensities of excitation the curve drops again after passing through a maximum. In this region increase of excitation no longer causes an increase in the reaction, but a decrease. Mozhaeva also showed that the curves of the local response may change their shape after the action of certain substances on the nerve.

Nasonov also thought it important to check the existence of gradual relationships in the separate conducting units, where the results would not be complicated by statistical laws. Studies on the isolated giant nerve fiber of the squid (Lev, Nikol'skii, Rozental', Shapiro, 1958a, 1958b, 1959) and the isolated muscle fiber of a frog (Krolenko, 1958) were devoted to this purpose.

In the former case a complete S-shaped curve was obtained, expressing the gradual relationship between intensity of excitation and magnitude of the electric response reaction. The laws of spreading of impulse—spatial decrement of subthreshold excitation, and incremental spread of excitation arising from the suprathreshold intensities of excitation following the gradually forming relationships, were also confirmed. The dependence curve between excitation and response in the case of a freshly
376 isolated fiber rises sharply, becoming less steep in the case of a fiber kept under observation for some time. This is accompanied by transition from a nondecremental conduction to conduction with decrement, as postulated by the theory of gradual excitation.

Similar changes in the response reaction after deterioration of the functional state were observed by Krolenko on an isolated tetanic muscle fiber of frog. A muscle fiber in an optimal functioning condition responds to a single electric excitation of varying intensity by practically the same amplitude of contraction waves. At the basis of this is the wave of excitation spreading along the entire fiber. After deterioration of the functional state due to various factors, the curve relating the magnitude of contraction to the intensity of excitation acquires a smooth S-form. The capacity for nondecremental conduction is lost.

Nasonov considered it necessary to conduct further experimental work on this theory. The laboratory of cell physiology at the Institute of Cytology of the Academy of Sciences of the USSR will continue to work in this direction.

D. L. Rozental'

BIBLIOGRAPHY

Publications in Russian

1. ABRIKOSOV, I. A. and V. N. DARKSHEVICH. Faktor vremeni pri otsenke vozbudimosti tkanei. (Po povodu stat'i D. N. Nasonova i D. L. Rozental') (The Time Factor in the Evaluation of Tissue Excitability. (Concerning the paper by D. N. Nasonov and D. L. Rozental')). – Fiziologicheskii Zhurnal SSSR, 40(4):504. 1954.
2. AFANAS'EV, P. V. and Yu. N. IL'INA. O faktorakh, vliyayushchikh na skorost' fermentativnykh protsessov i na aktivnost' fermentov (Factors Affecting the Velocity of Enzymatic Processes and the Activity of Enzymes). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 4:495. 1949.
3. AIZENBERG, E. I. Vliyanie temperatury na mitozy mnogosloinogo epiteliya kozhi lyagushki (Effect of Temperature on Mitoses in the Stratified Epithelium of the Frog Skin). – Arkhiv Anatomii, Gistologii, i Embriologii, 13(1): 115. 1934.
4. AIZENBERG, E. I. Vliyanie rastvorov neelektrolitov na soderzhanie vody v yaitsevykh kletkakh (Effect of Solutions of Nonelectrolytes on the Water Content of Egg Cells). –Arkhiv Anatomii, Gistologii i Embriologii, 22(1):86. 1939.
5. ALEKSANDROV, V. Ya. Vliyanie luchistoi energii na pronitsaemost' kletok i tkanei (Effect of Radiant Energy on the Permeability of Cells and Tissues). – Vestnik Rentgenologii i Radiologii, 13(6):470. 1934.
6. ALEKSANDROV, V. Ya. Denaturatsionnye izmeneniya belkov pri fiziologicheskikh protsessakh (Denaturation Changes in Proteins in Physiological Processes). – Uspekhi Sovremennoi Biologii, 24(4):44. 1947.
7. ALEKSANDROV, V. Ya. Spetsificheskoe i nespetsificheskoe v reaktsii kletki na povrezhdayushchie vozdeistviya (The Specific and Nonspecific Factors in the Reaction of the Cell to Noxious Agents). – Trudy Instituta Tsitologii, Gistologii i Embriologii, 3(1):3. 1948.
8. ALEKSANDROV, V. Ya. and N. I. ARRONET. Adenozintrifosfat vyzyvaet dvizhenie resnichek mertsatel'nogo epiteliya, ubitogo glitserinovoi ekstraktsiei (Kletochnaya model') (Adenosinetriphosphate Induces Movement of Cilia in a Glycerine-Extracted Ciliary Epithelium ("Cellular Model")). – Doklady Akademii Nauk SSSR, 110(3):457. 1956.
9. ALEKSANDROV, V. Ya. and L. G. VOL'FENZON. Obratimye sokrashcheniya soedinitel'notkannykh kletok pri deistvii razlichnykh agentov (Reversible Contractions of Connective Tissue Cells under the Influence of Various Agents). – Zhurnal Obshchei Biologii, 17(2):142. 1956.
10. ALEKSANDROV, V. Ya. and D. N. NASONOV. O prichinakh kolloidnykh izmenenii protoplazmy i uvelicheniya srodstva ee k krasitelyam pod vliyaniem povrezhdayushchikh vozdeistvii (The Causes of Colloid Changes in Protoplasm and Increase in its Affinity for Dyes under the Influence of Noxious Agents). – Arkhiv Anatomii, Gistologii i Embriologii, 22(1):11. 1939.
11. ALEKSANDROV, S. N. Skorost' rasprostraneniya povrezhdeniya v myshechnykh voloknakh razlichnogo diametra (Velocity of Spread of Damage in Muscle Fibers of Various Diameters). – Doklady Akademii Nauk SSSR, 63(3):337. 1948a.
12. ALEKSANDROV, S. N. Skorost' rasprostraneniya povrezhdeniya v myshtsakh lyagushek razlichnogo vozrasta (Velocity of Spread of Damage in Muscles of Frogs of Different Ages). – Doklady Akademii Nauk SSSR, 63(4):459. 1948b.
13. ALEKSANDROV, S. N. Temperaturnyi koeffitsient skorosti rasprostraneniya povrezhdeniya v somaticheskoi muskulature pozvonochnykh (Temperature Coefficient of the Velocity of Spread of Damage in the Body Musculature of Vertebrates). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 27(2):121. 1949.
14. ALEKSANDROV, S. N. O prichinakh, obuslovlivayushchikh svoeobrazie kharaktera irradiatsii povrezhdeniya v tonicheskikh voloknakh lyagushki (Causes of the Specific Nature of Irradiation Damage in Tonic Fibers of the Frog). – Doklady Akademii Nauk SSSR, 103(1):169. 1955.

15. ALEKSANDROV, S. N. and L. I. LEUSHINA. O nekotorykh chertakh skhodstva tonicheskikh i tetanicheskikh myshechnykh volokon. II soobshchenie (Certain Features of Similarity of Tonic and Tetanic Muscle Fibers. Communication II). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 35(1):7. 1953.

16. ANOKHIN, P. K., V. S. MAIORCHIK, and Ya. L. SLAVUTSKII. Primenenie ritmicheskikh razdrazhenii, kak novyi metod izucheniya i diagnostiki travmy nervnoi sistemy (The Use of Rhythmic Excitation as a New Method of Study and Diagnosis of Trauma of the Nervous System). – Voprosy Neirokhirurgii 9(1):54. 1945.

17. AVERBAKH, M. S. K istorii ucheniya ob akkomodatsii (History of the Accommodation Theory). – Vestnik Leningradskogo Universiteta, Vol. 7:69. 1948.

18. AVERBAKH, M. S. and D. N. NASONOV. Zakon samoregulyatsii rasprostranyayushchegosya vozbuzhdeniya ("vse ili nichego") (Law of Autoregulation of Spreading Excitation ("All or None")). – Fiziologicheskii Zhurnal SSSR, 36(1):46. 1950.

19. BARBASHOVA, Z. I. and A. G. GINETSINSKII. Vliyanie akklimatizatsii na prizhiznennuyu okrashivaemost' tkanei (Effect of Acclimatization on Vital Staining of Tissues). – Materialy po Evolyutsii Fiziologii, Vol. 1:36. 1956.

20. BARKHASH, A. P. Raspredelenie aminoazota mezhdu eritrotsitami i plazmoi pri eksperimental'noi anemii u krolikov (Distribution of Amino-Nitrogen between Erythrocytes and Plasma in Experimental Anemia of Rabbits). – Fiziologicheskii Zhurnal SSSR, 17(5):1078. 1934.

21. BELITSER, V. A. Denaturatsiya i svyazannye s nei izmeneniya belkov (Denaturation and Changes in Proteins Associated with it). – Uspekhi Biologicheskoi Khimii, Vol. 1:53. 1950.

22. BELITSER, V. A. Vzaimodeistvie belkovykh chastits pri spetsificheskikh reaktsiyakh. Belki, ikh spetsificheskie svoistva (Interaction between Protein Particles in Specific Reactions. Proteins and their Properties) p. 110. – Kiev. 1955

23. BERITOV, I. S. Obshchaya fiziologiya myshechnoi i nervnoi sistemy (General Physiology of the Muscular and Nervous Systems) pp. 1-155. – Moskva-Leningrad. 1947

24. BALDWIN, E. Osnovy dinamicheskoi biokhimii (Dynamic Aspects of Biochemistry) pp. 1-506. – Moskva. 1949. [Cambridge University Press, 1949].

25. BRAUN, A. A. and M. F. IVANOV. Vital'naya okraska poperechnopolosatoi myshechnoi tkani v razlichnykh eksperimental'nykh usloviyakh (Vital Staining of Striated Muscle Tissue under Various Experimental Conditions). – Arkhiv Anatomii, Gistologii i Embriologii, 12(1):3. 1933.

26. BRAUN, A. D. Svyazyvanie krasitelei nativnymi i denaturirovannymi belkami (Binding of Dyes by Native and Denaturated Proteins). – Biokhimiya, 13(5):409 1948a.

27. BRAUN, A. D. Svyazyvanie krasitelei nativnymi i denaturirovannymi nityami miozina (Binding of Dyes by Native and Denaturated Myosin Fibers). – Doklady Akademii Nauk SSSR, 62(2):263. 1948b.

28. BRAUN, A. D. Vzaimodeistvie nativnykh i denaturirovannykh belkov s krasitelyami (Interaction between Native and Denaturated Proteins and Dyes) Thesis. – Institut Eksperimental'noi Meditsiny Akademii Meditsinskikh Nauk SSSR. 1949.

29. BRAUN, A. D. Vzaimodeistvie belkov s trifenilmetanovymi krasitelyami (Interaction of Proteins with Triphenylmethane Dyes). – Biokhimiya, 16(5):399. 1951.

30. BRAUN, A. D. K voprosu o prichinakh, mekhanizme i klinicheskom znachenii kreatinurii (Causes, Mechanism and Clinical Importance of Creatinuria). – Tezisy Dokladov na Otchetnoi Nauchnoi Sessii Instituta Akusherstva i Ginekologii Akademii Meditinshikh Nauk SSSR, p. 10. 1955.

31. BRAUN, A. D. Vykhod kreatina iz skeletnykh myshts, nakhodyashchikhsya v pokoe i pod vliyaniem razlichnykh razdrazhitelsei (Passage of Creatine from Skeletal Muscles at Rest and under the Influence of Various Irritants). – Tezisy Dokladov Nauchnoi Konferentsii Instituta Tsitologii Akademii Nauk SSSR, Posvyashchennoi 40-i Godovshchine Velikoi Oktyabr'skoi Sotsialisticheskoi Revolyutsii, p. 3, Leningrad. 1958.

32. BRAUN, A. D., M. V. SAVOST'YANOVA, and R. I. MOROZOVA. Spektrofotometricheskoe izucheniye obestsvechivaniya trifenilmetanovykh krasitelei v shchelochnoi srede i v prisutstvii belka (Spectrophotometric Study of Decolorization of Triphenylmethane Dyes in an Alkaline Medium and in the Presence of Proteins). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 14:536. 1950.

33. BRAUN, A. D. and N. L. FEL'DMAN. Toksichnost' krasitelei i svyazyvanie ikh nativnymi belkami (Toxicity of Dyes and their Binding by Native Proteins). – Doklady Akademii Nauk SSSR, 68(4): 757. 1949.

34. BRIDGMAN P. W. Noveishie raboty v oblasti vysokikh davlenii (Latest Studies in the Field of High Pressures) pp. 1-297. – Moskva, 1948.

35. BRUSILOVSKAYA, A. I. Issledovaniya sorbtsii letuchikh narkotikov krov'yu. II. sorbtsiya parov toluola krov'yu (Studies of Sorption of Volatile Narcotics by the Blood. II. Sorption of Toluene Vapors by the Blood). – Farmakologiya i Toksikologiya, 2(3):31. 1939.

36. BRUSILOVSKAYA, A. I. Raspredelenie narkotikov mezhdu eritrotsitami i plazmoi (Distribution of Narcotics between Erythrocytes and Plasma). – Farmakologiya i Toksikologiya, 6(1):48. 1947.

37. BUTKEVICH, V. P. Deistvie termicheskogo razdrazhitelya na myshechnuyu tkan' lyagushki (Effect of Thermal Irritant on Muscle Tissue of the Frog). – Vestnik Leningradskogo Universiteta, No. 1:124. 1948.

38. CHAGOVETS, V. Yu. Ocherk electricheskikh yavlenii na zhivykh tkanyakh s tochki zreniya noveishikh fizikokhimicheskikh teorii (Outlines of Electric Phenomena in Live Tissues from the Point of View of the Latest Physicochemical Theories). No. 1, pp. 1-309. – St. Peterburg. 1903.

39. CHEREPANOVA, T. N. and I. P. SUZDAL'SKAYA. Sovmestnoe deistvie nekotorykh agentov na tkani kholodnokrovnykh zhivotnykh (Joint Action of Certain Agents on Tissues of Cold-Blooded Animals). – Vestnik Leningradskogo Universiteta, Vol. 1:91. 1954.

40. CHETVERIKOV, G. N. Stroenie nervno-myshechnykh okonchanii v tonicheskikh i netonicheskikh myshtsakh (Structure of Neuromuscular Endings in Tonic and Atonic Muscles). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 36(1):53. 1953.

41. DANZHAR, P. Tsitologiya rastenii i obshchaya tsitologiya (Cytology of Plants and General Cytology), pp. 1-652. – Moskva. 1950.

42. DEMIN, N. N. Raspredelenie aminoazota v krovi pri perekhode ee iz arterial'noi v venozmuyu (Distribution of Amino-Nitrogen in the Blood on Changing from Arterial into Venous Blood). – Byulleten' Eksperimental' noi Biologii i Meditsiny, 12(1):92. 1941a.

43. DEMIN, N. N. Aminokisloty i polipeptidy krovi pri dlitel'nom eksperimental'nom golodanii (Amino Acids and Polypeptides of the Blood in Prolonged Experimental Starvation). – Voprosy Pitaniya, 10(3/4):16. 1941b.

44. DZHAMUSOVA, T. A. Issledovanie mestnogo stoikogo vozbuzhdeniya myshtsy pri dlitel'nom deistvii na nee khloristogo natriya (Study of Local Stable Excitation of Muscle by Prolonged Action of Sodium Chloride). – Fiziologicheskii Zhurnal SSSR, 44(7):664. 1958.

45. DZHAMUSOVA, T. A. and V. V. PONOMARENKO. Sootnoshenie vozbudimosti i ritmicheskoi aktivnosti pri parabioze poperechnopolosatoi muskulatury (The Relationship between Excitability and Rhythmic Activity During Parabiosis of Striated Musculature). – Fiziologicheskii Zhurnal SSSR, 40(2):198. 1954.

46. EBBEKE, U. Szhatie myshtsy i ee sokrashchenie pod vliyaniem vysokogo davleniya (Compression and Contraction of Muscle under the Influence of High Pressure). – XV Mezhdunarodnyi Fiziologicheskii Kongress, Tezisy Soobshchenii, p. 467, Moskva-Leningrad. 1935.

47. ENGEL'GARDT, V. A. Fermentativnye i mekhanicheskie svoistva belkov myshtsy (Enzymatic and Mechanic Properties of Muscle Proteins). – Uspekhi Sovremennoi Biologii, 2(14):177. 1941.

48. ENGEL'GARDT, V. A. and A. I. KOLOTILOVA. Usloviya glikoliza v eritrotsitakh razlichnykh zhivotnykh (Conditions for Glycolysis in Erythrocytes of Various Animals). – Trudy Fiziologicheskogo Instituta Leningradskogo Universiteta, Vol. 16:3. 1936.

49. EPSHTEIN, Ya. A. Priroda organicheskikh kislot mozgovoi tkani (Nature of Organic Acids in Brain Tissue). – VII Vsesoyuznyi S"ezd Fiziologov, Biokhimikov i Farmakologov, Doklady, p. 666, Moskva. 1947.

50. FEL'DMAN, N. L. K voprosu o diffuznom okrashivanii kletki nekotorymi osnovnymi vital'nymi krasitelyami (Diffuse Staining of the Cell by Certain Basic Vital Stains). – Doklady Akademii Nauk SSSR, 54(6): 1173. 1948a.

51. FEL'DMAN, N. L. Rol'koatservatsii v otlozhenii granul osnovnykh vital'nykh krasitelei v kletke (The Role of Coacervation in the Deposition of Granules of Basic Vital Stains in the Cell). – Doklady Akademii Nauk SSSR, 62(6):817. 1948b.

52. FEL'DMAN, N. L. Vliyanie denaturatsii belkov na koatservatsiyu ikh s krasitelyami (Effect of Protein Denaturation on their Coacervation with Dyes). – Doklady Akademii Nauk SSSR, 74(6):1139. 1950.

53. FEL'DMAN, N. L. O prichinakh podavleniya granulootlozheniya krasitelya pri povrezhdenii kletok (The Causes of Suppression of Granule Formation of Dye during Cellular Damage). – Doklady Akademii Nauk SSSR, 89(2):345. 1953.

54. FRENKEL' Ya. I. Relaksatsionnaya teoriya slukha (Relaxation Theory of Hearing). – Doklady Akademii Nauk SSSR, 59(4):679. 1948.

55. FRIDLYAND, I. B. Aminnyi i polipeptidnyi azot krovi i raspredelenie ego mezhdu eritrotsitami i plazmoi pri eksperimental'nom skorbute u morskikh svinok (Amine and Polypeptide Nitrogen of the Blood and its Distribution between Erythrocytes and Plasma in Experimental Scurvy in Guinea Pigs). – Fiziologicheskii Zhurnal SSSR, 27(2):244. 1939.

56. GABRICHEVSKII, G. O vozbudimosti myshts v zavisimosti ot fizicheskikh i khimicheskikh svoistv miozina (Excitability of Muscles as a Function of the Physical and Chemical Properties of Myosin). – Trudy Fiziologicheskoi Laboratorii Moskovskogo Universiteta, Vol. 1:1-140. 1888.

57. GAVRILOVA, L. Kontraktury poperechnopolosatykh myshts, poluchennye deistviem gipotonicheskikh rastvorov (Contractions of Striated Muscles Under the Influence of Hypotonic Solutions). – Doklady Akademii Nauk, SSSR, 63(5):589. 1948.

58. GARVEI, E. and D. DANIELLI. Poverkhnost' kletki i ee svoistva (The Surface of the Cell and its Properties). – Uspekhi Sovremennoi Biologii, 10(3):471. 1939.

59. GEL'KHORN, E. Problema pronitsaemosti, ee fiziologicheskoe i patologicheskoe znachenie (The Problem of Permeability, its Physiological and Pathological Importance), pp. 1-336, Moskva. 1932.

60. GENNI, M. M. Materialy k probleme evolyutsii somaticheskogo myshechnogo volokna (Data on the Evolution of Somatic Muscle Fibers). Thesis. – Fiziologicheskii Institut Akademii Nauk SSSR, Leningrad. 1947.

61. GERMAN, L. Rukovodstvo k fiziologii. Obshchaya myshechnaya fizika (Textbook of Physiology. Vol. 1, part 1. Physical Properties of Muscle), pp. 1-384. – St. Peterburg. 1885.

62. GERMAN, L. Rukovodstvo k fiziologii, Tom 2, chast' 1. Obshchaya nervnaya fiziologiya (Textbook of physiology. Vol. 2, part 1. General Nerve Physiology), pp.1-394. – St. Peterburg. 1889.

63. GINETSINSKII, A.G. Rol' atsetilkholina v protsesse provedeniya vozbuzhdeniya (The Role of Acetylcholine in the Process of Conduction of Excitation). Gagry Symposium, 1, p. 109. – Tbilisi. 1949.

64. GINETSINSKII, A.G. and Z.I. BARBASHOVA. Po povodu novoi gipotezy o fiziologicheskom znachenii atsetilkholina (A New Hypothesis on the Physiological Importance of Acetylcholine). – Trudy Fiziologicheskogo Instituta im Pavlova Akademii Nauk SSSR, Vol. 4:149. 1949.

65. GOLIKOV, N.V. Izmeneniya khronaksii i refrakternoi fazy nerva pod vliyaniem tekushchikh impul'sov (Changes in Chronoxy and Refrectory Phase of the Nerve Under the Influence of Passing Impulses). – Trudy Fiziologicheskogo Instituta Leningradskogo Universiteta, Vol. 14:20. 1934.

66. GOLIKOV, N.V. O mekhanizmakh subordinatsionnykh vliyanii (Mechanisms of Subordinational Influences). – Trudy Leningradskogo Obshchestva Estestvoispytatelei, 69(5):16. 1950.

67. GOLOVINA, N.V. Solevye toki zhivykh myshts i myshts, zafiksirovannykh formalinom (Salt Currents in Living Muscles and in Formalin-Fixed Muscles). – In Sbornik: "Pamyati akademika A. A. Zavarzina", p. 529, Moskva-Leningrad. 1948.

68. GOLOVINA, N.V. Substantsional'nye izmeneniya nervnogo volokna pri vozbuzhdenii (Substantial Changes in the Nerve Fiber on Stimulation). – Thesis. Institut Eksperimental'noi Meditsiny Akademii Meditsinskikh Nauk SSSR, 1949.

69. GOLOVINA, N.V. Deistvie vysokogo gidrostaticheskogo davleniya na poperechnopolosatuyu muskulaturu lyagushki (Effect of High Hydrostatic Pressure on the Striated Muscles of the Frog). – Doklady Akademii Nauk SSSR, 105(6):1378. 1955a.

70. GOLOVINA, N.V. Sorbtsionnye svoistva tserebro-vistseral'noi konnektivy bezzubki (Sorptional Properties of the Cerebro-Visceral Connective of the Fresh-Water Mussel). – Fiziologicheskii Zhurnal SSSR, 41(6):822. 1955b.

71. GOLOVINA, N.V. Faza snizheniya sorbtsionnogo urovnya i vozbudimost' myshtsy (The Phase of Decrease in the Sorption Level and Muscle Excitability). Manuscript. – Institut Tsitologii Akademii Nauk SSSR. 1958a.

72. GOLOVINA, N.V. Deistvie vysokogo gidrostaticheskogo davleniya na izolirovannyi mozg beloi myshi (Effect of High Hydrostatic Pressure on the Isolated Brain of a White Mouse). – Manuscript. Institut Tsitologii Akademii Nauk SSSR. 1958b.

73. GRAMENITSKII, E.M. Rasprostranenie mekhanicheskogo povrezhdeniya v myshtsakh rechnogo raka (Spread of Mechanical Damage in Muscles of the Fresh Water Crab). – In Sbornik: "Pamyati Akademika A.A. Zavarzina", p. 476, Moskva-Leningrad. 1948.

74. GRAMENITSKII, E.M. O nekotorykh usloviyakh vozniknoveniya i rasprostraneniya mekhanicheskogo po povrezhdeniya v skeletnoi myshtse lyagushki (Certain Conditions of Formation and Spread of Mechanical Damage in Skeletal Muscle of the Frog). – Avtoreferat Dissertatsii pp. 1-8. Leningrad. 1949.

75. GRINDEL', O.M. and V.S. RUSINOV. Deistvie postoyannogo toka na begushchuyu volnu v al'terirovannom nerve (Effect of Direct Current on a Running Wave in an Altered Nerve). Gagry Symposium 1, p. 278. – Tbilisi. 1949.

76. GULINOVA, E.I. Vliyanie okhlazhdeniya na funktsii nerva (Effect of Cooling on the Functions of the Nerve). – Raboty Fiziologicheskoi Laboratorii Universiteta, St. Peterburg, Vol. 1:411, 1906.

77. GUR'EVA, I.P. Raspredelenie aminnogo i polipeptidnogo azota mezhdu eritrotsitami i plazmoi pri tuberkuleze (Distribution of Amine and Polypeptide Nitrogen Between Erthrocytes and Plasma in Tuberculosis). Thesis. – Moskva. 1940.

78. GZHATSKII, S.Z. and F.I. VANDOKANTI. Sintez glikogena v pecheni i muskulakh pri vlivanii glyukozy i s pribavleniem adrenalina i insulina (Synthesis of Glycogen in the Liver and Muscles from Glucose and with the Addition of Adrenalin and Insulin). – Sbornik Nauchnykh Trudov L'vovskogo Veterinarnogo Instituta, Vol. 1:127. 1947.

79. HILL, A. Etyudy iz oblasti biofiziki (Studies in Biophysics) pp. 1-164. – Moskva-Leningrad. 1935.

80. HEVESY, G. Radioaktivnye indikatory (Radioactive Tracers), pp. 1-540. – Moskva. 1950.

81. IL'IN, V.S. and V.V. TIKHAL'SKAYA. O vykhode ionov fosfornoi kisloty iz pokoyashcheisya i rabotayushchei myshtsy (Passage of Phosphoric Acid Ions from Muscles at Rest and during Activity). – Fiziologicheskii Zhurnal SSSR, 14(2/3):289. 1931.

82. IL'INSKAYA, N.B. and B.P. USHAKOV. Osobennosti solevogo parabioza (mestnogo vozbuzhdeniya) retraktorov Phascolosoma margaritaceum (Properties of Salt Parabiosis [local excitation] of the retractors of Phascolosoma margaritaceum). – Doklady Akademii Nauk SSSR, 83(6):961. 1952.

83. INDZHIKYAN, A.T. Fiziologicheskii pokoi i tormozhenie myshtsy v zavisimosti ot solevogo sostava ee sredy (Physiological Rest and Blocking of a Muscle as a Function of the Salt Composition of the Medium). – Trudy Fiziologicheskogo Instituta Leningradskogo Universiteta, Vol. 18:71. 1937.

84. KAMNEV, I.E. Izmeneniya epitelial'nykh kletok zhivotnykh pod vozdeistviem destillirovannoi vody i solevykh rastvorov Ringera (Changes in Epithelial Cells of Animals under the Influence of Distilled Water and of Ringer's Salt Solutions). – Trudy Fizologicheskogo Instituta Lenigradskogo Universiteta, Vol. 14:151. 1934a.

85. KAMNEV, I.E. Vliyanie izotonicheskogo rastvora sakhara na kletki razlichnykh tkanei amfibii (Effect of an Isotonic Sugar Solution on Cells of Different Amphibian Tissues). – Trudy Fiziologicheskogo Instituta Leningradskogo Universiteta, Vol. 16:111. 1936.

86. KAMNEV, I.E. Pronitsaemost' poperechnopolosatykh myshts lyagushki dlya sakharov (Permeability of Striated Muscles of the Frog to Sugars). – Arkhiv Anatomii, Gistologii i Embriologii, 19(1/2):145. 1938.

87. KAMNEV, I.E. Substantsional'nye izmeneniya protoplazmy pod vliyaniem elektricheskogo toka (Substantial Changes in the Protoplasm under the Influence of Electric Current). – Trudy Instituta po Izucheniyu Mozga im V.M. Bekhtereva, Vol. 14:48. 1941.

88. KAMNEV, I.E. Ritmicheskie volny sokrashcheniya, vyzyvaemye v skeletnoi myshtse deistviem postoyannogo elektricheskogo toka (Rythmic Waves of Contraction Caused in Skeletal Muscle by Direct Current). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 4:437. 1948a.

89. KAMNEV, I.E. Issledovanie reaktsii zhivoi protoplazmy., vyzyvaemoi deistviem postoyannogo elektricheskogo toka (Investigation of the Reaction of Living Protoplasm to a Direct Current). – Byuletten' Eksperimental'noi Biologii i Meditsiny, 25(6):459. 1948b.

90. KAMNEV, I.E. O nekotorykh substantsional'nykh i funktsional'nykh izmeneniyakh skeletnoi myshtsy, voznikayushchikh pod vliyaniem razlichnykh eksperimental'nykh vozdeistvii (Some Substantial and Functional Changes in Skeletal Muscle Caused by Various Experimental Procedures). – In: Sbornik "Pamyati Akademika A.A. Zavarzina", p. 493, Moskva-Leningrad. 1948c.

91. KAMNEV I.E. Reaktsiya rogovoi obolochki glaza na deistvie postoyannogo elektricheskogo toka (Reaction of the Corneal Membrane of the Eye to Direct Current). – Zhurnal Obshchei Biologii, 10(2):4. 1949.

92. KAN, I.L. Vystuplenie na konferentsii po probleme pronitsaemosti (Short Report Presented at the Conference on Permeability). – In Sbornik: "Problema pronitsaemosti", p. 109. Trudy Konferentsii Moskovskogo Obshchestva Fiziologov. 1939.

93. KAN, I.L. and D.P. KUZNETSOV. O dekrementnom provedenii vozbuzhdeniya v bezmyakotnoi nervnoi komissure anodonta (Decremental Conduction of Excitation in the Nonmedullated Nervous Comissure of Anodonta). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 6(1):3. 1938.

94. KAPLANSKII, S. Ya. Mineral'nyi obmen (Metabolism of Minerals), pp. 1-312. – Moskva, 1938.

95. KAPLANSKII, S.Ya. and M. BOLDYREVA. K voprosu o regulyatsii mineral'nogo obmena u gomoosmoticheskikh ryb pri razlichnom mineral'nom sostave vody (On the Problem of Regulation of Metabolism of Minerals in Homoosmotic Fishes at Various Compositions of Minerals in the Water). – Fiziologicheskii Zhurnal SSSR, 16(1):219. 1933.

96. KAPLANSKII, S. Ya., and M. BOLDYREVA. K voprosu o regulyatsii mineral'nogo obmena u gomoosmoticheskikh ryb pri izmenenii mineral'nogo sostava vody (On the Problem of Regulation of Metabolism of Minerals in Homoosmotic Fishes with Changes in the Mineral Composition of the Water). – Fiziologicheskii Zhurnal SSSR, 17(1):96. 1934.

97. KARAEV, A. I. Znachenie uglevodov dlya elektrogenii myshts (Importance of Carbohydrates for Electrogenesis in Muscles). – Fiziologicheskii Zhurnal SSSR, 23(2): 241. 1937.

98. KARAEV, A. I. O predelakh rasprostraneniya lokalnykh podporogovykh izmenenii v nerve (Limits of Spreading of Local Subthreshold Changes in the Nerve). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 6(1): 7. 1938.

99. KARASIK, V. M. Farmakologicheskii analiz avtomatii (Pharmacological Analysis of Automatic Action). – Fiziologicheskii Zhurnal SSSR, 33(4): 463. 1947.

100. KHODOROV, B. I. Vliyanie monoioduksusnoi kisloty na vozbudimost' i akkomodatsiyu nerva lyagushki (Effect of Monoiodoacetic Acid on Excitability and Accommodation of the Frog Nerve). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 27(1): 50. 1949.

101. KHODOROV, B. I. Issledovanie mekhanizma i prirody akkomodatsii nerva (Investigation of the Mechanism and Nature of Nerve Accommodation). Author's Summary, pp. 1-30. – Moskva. 1950a.

102. KHODOROV, B. I. O kazhushchikhsya i istinnykh izmeneniyakh vozbudimosti nerva na polyusakh postoyannogo toka (Apparent and Real Changes in Excitability of the Nerve at the Poles of Direct Current). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 29(4): 272. 1950b.

103. KHODOROV, B. I. O vliyanii khloristogo kaliya na vozbudimost' i akkomodatsiyu nerva lyagushki (Effect of Potassium Chloride on Excitability and Accommodation of the Frog Nerve). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 29(5): 339. 1950c.

104. KHODOROV, B. I. Vliyanie tsianidov na vozbudimost' i akkomodatsiyu normal'nogo i otravlennogo monoiodatsetatom nerva (Effects of Cyanides on Excitability and Accommodation of a Normal and Monoiodoacetate-Poisoned Nerve). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 29(6): 425. 1950d.

105. KHODOROV, B. I. Elektroton i akkomodatsiya (Electrotonus and Accommodation). – Uspekhi Sovremennoi Biologii, 29(3): 329. 1950e.

106. KHODOROV, B. I. Vydayushchiisya russkii fiziolog B. F. Verigo (1860-1925) (The Outstanding Russian Physiologist B. F. Verigo (1860-1925)). – Zhurnal Obshchei Biologii, 12(1): 55. 1951.

107. KIRO, M. B. Vital'naya okraska vozbuzhdennykh myshts (Vital Staining of Stimulated Muscles). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 4: 419. 1948.

108. KIRO, M. B. Uvelichenie vremeni perezhivaniya izolirovannykh myshts lyagushki pri deistvii nekotorykh khimicheskikh agentov (Prolongation of Survival Time of Isolated Frog Muscles under the Influence of Certain Chemical Agents). – Vestnik Leningradskogo Universiteta, Vol. 1: 111. 1954.

109. KISELEV, P. A. O znachenii faktora vremeni v kharakteristike vozbudimosti. (Po povodu stat'i D. N. Nasonova i D. L. Rozental') (Importance of the Time Factor in the Characterization of Excitability. (In response to the paper by D. N. Nasonov and D. L. Rozental')). – Fiziologicheski Zhurnal SSSR, 40(4): 510. 1954.

110. KOLOTILOVA, A. I. Rol' pronitsaemosti v yavleniyakh glikoliza (Role of Permeability in the Phenomena of Glycolysis). – Trudy Fiziologicheskogo Instituta Leningradskogo Universiteta, Vol. 19: 121. 1937.

111. KOLOTILOVA, A. I. and V. A. ENGEL'GARDT. Pronitsaemost', glikoliz i raspredelenie sakhara v eritrotsitakh (Permeability, Glycolysis and Distribution of Sugar in Erythrocytes). – Biokhimiya 2(2): 387. 1937.

112. KOMETIANI, P. A. O svyazi mezhdu atsetilkholinom i kaliem v myshechnoi tkani (On the Relationship between Acetylcholine and Potassium in Muscle Tissue). – VIII Vsesoyuznyi S"ezd Fiziologov, Biokhimikov i Farmakologov, Doklady, p. 340. Moskva. 1947.

113. KOMETIANI, P. A. Svyaz' mezhdu atsetilkholinom i kaliem v myshechnoi tkani (The Relationship between Acetylcholine and Potassium in Muscle Tissue). – Trudy Instituta Fiziologii im. I. Beritashvili Akademii Nauk Gruzinskoi SSSR, Vol. 7: 393. 1948a.

114. KOMETIANI, P. A. Issledovanie vliyaniya atsetilkholina na ravnovesie kaliya s rastvorennymi belkami myshechnoi tkani (Effect of Acetylcholine on the Equilibrium between Potassium and Soluble Proteins of Muscle Tissue). – Biokhimiya, 13, 2: 137. 1948b.

115. KOMETIANI, P. A. Svyaz' metabolicheskikh protsessov s biotokom (Connection between Metabolic Processes and Biocurrent). – Gagry Symposium 1, p. 51, Tbilisi. 1949.

116. KOMETIANI, P. A., Sh. V. DOLIDZE and E. E. KLEIN. Izmeneniya raspredeleniya kaliya v myshechnoi tkani pod vliyaniem atsetilkholina (Changes in Distribution of Potassium in Muscle Tissue under the Influence of Acetylochline). – Biokhimiya, 9(3): 218. 1944.

117. KOMETIANI, P. A., E. E. KLEIN and Sh. V. DOLIDZE. O mekhanize deistviya atsetilkholina na myshechnuyu tkani (Mechanism of Action of Acetylcholine on Muscle Tissue). – Biokhimiya, 11(3): 233. 1946.

118. KOMKOVA, A. I. and B. P. USHAKOV. Temperaturnaya inaktivatsiya adenozintrifosfatazy myshts travyanoi i ozernoi lyagushek (Temperature Inactivation of ATP-ase from Muscles of Grass and Lake Frogs). – Doklady Akademii Nauk SSSR, 102(6):1185. 1955.

119. KONDRASHOVA, M.N. K biokhimicheskoi kharakteristike parabioticheskogo protsessa (Biochemical Characterization of the Parabiotic Process). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 37(1):40. 1954.

120. KONIKOVA, A.S. Itogi i perspektivy izucheniya belkovogo obmena izotopnym metodom (Summary and Perspectives of the Study of Protein Metabolism by the Isotope Method). – In:Sbornik "Soveshchanie po Belku", p. 225, Moskva-Leningrad. 1948.

121. KORNAKOVA, E. V., G. M. Frank and L. N. SHTEINGAUZ. O strukturnykh protsessakh v nerve (Structural Processes in the Nerve). – Fiziologicheskii Zhurnal SSSR, 33(4):483. 1947.

122. KOTLYAREVSKAYA. M. A. and V. B. BOLDYREV. Opyt izucheniya nervnoi kletki (Experience from Studies of the Nerve Cell). – In Sbornik: "Morfologiya avtonomnoi nervnoi sistemy", p. 79, Moskva. 1939.

123. KOSHTOYANTS, Kh. S. Analiz putei deistviya atsetilkholina kak khimicheskogo faktora nervnogo vozbuzhdeniya (Analysis of the Manner of Action of Acetylcholine as the Chemical Factor in Nerve Stimulation). – Doklady Akademii Nauk SSSR, 43(8):376. 1944.

124. KOSHTOYANTS, Kh. S. Sravnitel'nye issledovaniya ob enzimokhimicheskoi prirode nervnogo vozbuzhdeniya i vyvody iz nikh (Comparative Studies of Enzymatic-Chemical Nature of Nerve Stimulation and Conclusions Derived from These Studies). – VII Vsesoyuznyi S'ezd Fiziologov, Biokhimikov i Farmakologov, Doklady, p. 343, Moskva. 1947.

125. KOSHTOYANTS, Kh. S. Belkovye tela, obmen veshchestv i nervnaya regulyatsiya (Protein Substances, Metabolism and Nervous Regulation), pp. 1-100. Moskva. 1951.

126. KOSHTOYANTS, Kh. S. Sbornik rabot Laboratorii obshchei i sravnitel'noi fiziologii. (Collection of Papers from Laboratory of General and Comparative Physiology). – Trudy Instituta Morfologii Zhivotnykh, Vol. 6: 1-185. 1952.

127. KRASIL'NIKOVA, V. I. K metodike opredeleniya funktsional'nogo sostoyaniya tkanei po kolichestvu svyazannogo krasitelya (Methods of Determining the Functional State of Tissues by the Amount of Bound Dye). – Fiziologicheskii Zhurnal SSSR, 40(4):476. 1954.

128. KROLENKO, S. A. Vliyanie mezhelektrodnogo rasstoyaniya na vozbudimost' izolirovannogo myshechnogo volokna (Effect of Interelectrode Distance on Excitability of an Isolated Muscle Fiber). – Biofizika, 1(5):401. 1956.

129. KROLENKO, S. A. Issledovaniya sokrashcheniya zolirovannogo myshechnogo volokna (Investigation of Contraction of an Isolated Muscle Fiber). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 3:313. 1958.

130. KULAKOVA, A. F. Raspredelenie nekotorykh aminokislot mezhdu eritrotsitami i plazmoi krovi (Distribution of Some Aminoacids between Erythrocytes and Blood Plasma). Thesis. – Moskva. 1939.

131. KURSANOV, A. L. Primenenie metoda vakuum-infil'trastii dlya kolichestvennogo opredeleniya sinteziruyushchego i gidroliziruyushchego deistviya invertazy v zhivykh rastitel'nykh kletkakh (Application of the Vacuum-Infiltration Method for Quantitative Determination of the Synthesis and Hydrolyzing Action of Invertase in Living Plant Cells). – Biokhimiya, 1(3):269. 1936.

132. KURSANOV, A. L. Obratimoe deistvie fermentov v zhivoi rastitel'noi kletke (Reversible Action of Enzymes in the Living Plant Cell), pp. 1-234. – Moskva-Leningrad. 1940.

133. KVASOV, D. G. Mnozhestvennyi otvet nerva na postoyannoe razdrazhenie (fenomen Rittera i Pflugera) (Multiple Response of the Nerve to Constant Excitation (Phenomenon of Ritter and Pflüger)). – Uspekhi Sovremennoi Biologii, 7(1):67. 1937.

134. KVASOV, D. G. Ob usloviyakh vozniknoveniya paradoksal'noi reaktsii skeletnoi myshtsy na ritmicheskoe razdrazhenie (Conditions for Appearance of a Paradoxical Reaction in Skeletal Muscle to Rythmic Excitation). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 27(3):183. 1949.

135. KVASOV, D. G. and A. I. NAUMENKO. Narusheniya izolirovannogo provedeniya impul'sov pri al'teratsii nerva vodo-otnimayushchimi faktorami (Disturbances of Isolated Conduction of Impulses During Alteration of the Nerve by Dehydrating Factors). – Fiziologicheskii Zhurnal SSSR, 20(6): 669. 1936.

136. KVASOV, D. G. and O. F. USHINSKAYA. Sluchai pryamogo vzaimodeistviya volokon skeletnoi myshtsy. (Elektrofiziologicheskii analiz) (A Case of Direct Interaction of Fibers of a Skeletal Muscle (Electrophysiological Analysis)). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 26(1):3. 1948.

137. LAZAREV, N. V. Biologicheskoe deistvie gazov pod davleniem (Biological Effect of Gases under Pressure), pp. 1–218. –Leningrad. 1941.

138. LAZAREV, N. V. and E. I. Nusel'man. O raspredelenii khloroforma mezhdu eritrotsitami i syvorotkoi (ili plazmoi) krovi (Distribution of Chloroform between Erythrocytes and the Blood Serum (or plasma)). –Fiziologicheskii Zhurnal SSSR, 15, (1/2): 126. 1932.

139. LAZAREV, P. P. Khronaksiya i ee rol' v klinicheskoi meditsine (Chronaxy and its Role in Clinical Medicine). –Klinicheskaya Meditsina, 12 (9): 1219. 1934.

140. LAZAREV, P. P. Sovremennye biofizicheskie metody issledovaniya v meditsine (Modern Biophysical Methods of Study used in Medicine). –Trudy Gosudarstvennogo Nauchno-Issledovatel'skogo Instituta Fizioterapii, Vol. 4: 15. 1939.

141. LAZAREV, P. P. Issledovaniya po adaptatsii (Studies on Adaptation), p. 63. –Moskva-Leningrad. 1947.

142. LAPIK, L. Noveishie uspekhi v poznanii nervnogo mekhanizma (Recent Advance in the Study of Nervous Nechanisms). –Fiziologicheskii Zhurnal SSSR, 21(5/6): 1059. 1936.

143. LEB, Zh. Dinamika zhivogo veshchestva (Dynamics of Living Matter), pp. –1–352. –Odessa. 1910.

144. LEBEDINSKII, A. V. Bioelektricheskie yavleniya i ikh ob'yasnenie s tochki zreniya membrannoi teorii (Bioelectric Phenomena and their Explanation from the Point of View of the Membrane Theory). –In Sbornik: "Problema pronitsaemosti", Trudy Konferentsii Moskovskogo Obshchestva Fiziologov, p. 75. Moskov 1935.

145. LEBEDINSKII, A. V., and A. S. Mozzhukhin. K voprosu o proiskhozhdenii bioelektricheskikh yavlenii (On the Origin of Bioeletric Phenomena). –Trudy Vsesoyuznoi Meditsinskoi Akademii im. Kirova, Vol 45: 5. 1950.

146. LEUSHINA, L. I., and S. N. Aleksandrov. Reaktsiya odinochnykh tonicheskikh i tetanicheskikh volokon na mekhanicheskoe povrezhdenie. Soobshchenie I (Reaction of Single Tonic and Tetanic Fibers to Mechanical Damage. Communication I). –Byulleten' Eksperimental'noi Biologii i Meditsiny, 35 (3): 20. 1953.

147. LEV, A. A., N. N. NIKOL'SKII, D. L. ROZENTAL' and E. A. SHAPIRO. Issledovanie elektricheskikh reaktsii v gigantskikh odinochnykh nervnykh voloknakh tikhookeanskogo kal'mara (Investigation of Electric Reactions in Giant Single Nerve Fibers of the Pacific Ocean Squid). –Tezisy Dokladov Nauchnoi Konferentsii Instituta Tsitologii Akademii Nauk SSSR, Posvyashchennoi 40-i Godovshchine Velikoi Oktyabr'skoi Sotsialisticheskoi Revolyutsii, p. 14. Leningrad. 1958a.

148. LEV, A. A., N. N. NIKOL'SKII, D. L. ROZENTAL' and E. A. SHAPIRO. Dekrementnoe i inkrementnoe provedenie v odinochnom nervom volokne kal'mara (Decremental and Incremental Conduction in a Single Nerve Fiber of the Squid). –Manuscript; Institut tsitologii Akademii Nauk SSSR. 1958b.

149. LEV, A. A., N. N. NIKOL'SKII, D. L. ROZENTAL', and E. A. SHAPIRO. Issledovanie zavisimosti mezhdu intensivnost'yu razdrazheniya i elektricheskoi otvetnoi reaktsiei v odinochnom nervnom volokne kal'mara (Investigation of the Relationship between Intensity of Excitation and Electrical Response Reaction in a Single Nerve Fiber of the Squid). –Tsitologiya, Vol. 1: 94. 1959.

150. LEV, A. A. and D. L. ROZENTAL'. Izmeneni sorbtsii krasitelya spinal'nymi gangliyami lyagushki pri razdrazhenii v zavisimosti ot funktsional'nogo sostoyaniya (Change of Sorption of Dye by Spinal Ganglia of the Frog upon Excitation Depending on the Functional State). –Biofizika, 3, 4: 413. 1958.

151. LEVIN, S. V. Vliyanie razdrazheniya interotseptorov na sorbtsionnye svoistva nervnykh kletok kory golovnogo mozga (Effect of Excitation of Interoceptors on the Sorptional Properties of the Nerve Cells of the Cerebral Cortex). –Byulleten' Eksperimental'noi Biologii i Meditsiny, 33 (4): 36. 1952.

152. LOZINA-LOZINSKII, A. L. Fotodinamicheskoe deistvie krasitelei na poperechnopolosatuyu muskulaturu lyagushki (Photodynamic Effect of Dyes on Striated Frog Muscles). –Vestnik Leningradskogo Universiteta, Vol. 7: 67. 1955.

153. LOPATINA, N. G., B. P. USHAKOV, and E. A. SHAPIRO. Sootnoshenie strukturnykh i funktsional'nykh izmenenii pri mestnom stoikom vozbuzhdenii myshts u bespozvonochnykh (The Relationship between Structural and Functional Changes in Local Stable Excitation of Muscles of Invertebrates). –Vestnik Leningradskogo Universiteta, Vol. 1: 85. 1953.

154. LYUDKOVSKAYA, R. G. Strukturnye protsessy v nervnoi tkani anodonty (Structural Processes in Nervous Tissue of Anodonta). –Doklady Akademii Nauk SSSR, 87 (5): 731. 1952.

155. LYUDKOVSKAYA, R. G. and G. M. FRANK. Opticheskie izmeneniya v mielinovom nerve pri razdrazhenii (Optical Changes in the Myelinated Nerve upon Excitation). –Doklady Akademii Nauk SSSR, 87 (3): 389. 1952.

156. MAGNITSKII, A. N. Problema subordinatsii (The Problem of Subordination). –In Sbornik "Subordinatsiya v nervnoi sisteme i ee znachenie v fiziologii i patologii", p. 5. Moskva. 1948.

157. MAGNITSKII, A. N. and V. A. MUZHEEV. Parabioz i khronaksiya (Parabiosis and Chronaxy). –Trudy Fiziologicheskogo Otdeleniya Gosudarstvennogo Timiryazevskogo Nauchno-Issledovatel'skogo Instituta, p. 77. Moskva. 1930.

158. MAKARAOV, P. V. K voprosu o vliyanii narkoticheskikh veshchestv na epitelial'nye kletki (Effect of Narcotics on Epithelial Cells). –Arkhiv Anatomii, Gistologii i Embriologii, 13 (1): 41. 1934.

159. MAKAROV, P. V. Vital'nye izmeneniya nervnykh kletok pod vliyaniem narkotikov (Vital Changes in Nerve Cells under the Influence of Narcotics). –Arkhiv Anatomii, Gistologii i Embriologii, 15 (4): 3. 1936a.

160. MAKAROV, P. V. Eksperimental'nye issledovaniya nad prosteishimi v svyazi s problemoi narkoza (Experimental Studies on Protozoa in Connection with the Problem of Narcosis). –Trudy Fiziologicheskogo Instituta Leningradskogo Universiteta, Vol. 16: 95. 1936b.

161. MAKAROV, P. V. Problema obshchego i kletochnogo narkoza (General and Cellular Narcosis). –Arkhiv Anatomii, Gistologii i Embriologii, 19 (1/2): 5. 1938.

162. MAKAROV, P. V. Izmenenie stroeniya yader ganglioznykh kletok pod vliyaniem razdrazhitelei (Change in Structure of Nuclei of Ganglial Cells under the Influence of Irritants). –Doklady Akademii Nauk SSSR, 61 (2): 353. 1948a.

163. MAKAROV, P. V. Fiziko-khimicheskie svoistva kletki i metody ikh izucheniya (Physicochemical Properties of Cells and Methods for their Investigation), pp. 1–324. –Leningrad. 1948b.

164. MAKAROV, P. V. Predislovie k knige: P. Danzhar. Tsitologiya rastenii i obshchaya tsitologiya (Introduction to the Book by P. Danzhar: Cytology of Plants and General Cytology). pp. 5–42. –Moskva. 1950.

165. MAKAROV, P. O. K kritike teorii provedeniya nervnogo impul'sa (Critical Evaluation of the Theories of Conduction of a Nerve Impulse). –Fiziologicheskii Zhurnal SSSR, 15 (1/2): 143. 1932.

166. MAKAROV, P. O. Dinamika vozbudimosti provedeniya i refrakternogo sostoyaniya. (Osnovnye elementy neirodinamiki) (Dynamics of Excitability of Conduction and of the Refractory State (Basic Elements of Neurodynamics)). –Trudy Leningradskogo Obshchestva Estestvoisplytatelei, 67 (1): 1. 1939.

167. MAKAROV, P. O. Bioelektricheskie toki pri mgnovennom povrezhdenii (Bioelectric Currents in Instantaneous Damage). Gagry Symposium 1, p. 195. –Tbilisi. 1949.

168. MAKSIMOV, N. A. Rostovye veshchestva, priroda ikh deistviya i prakticheskoe primenenie (Growth Substances, the Nature of their Action and their Practical Application). –Uspekhi Sovremennoi Biologii, 22 (2): 160. 1946.

169. MAKSIMOV, N. A. and L. V. MOZHAEVA. Vozrastnye izmeneniya kolloidno-khimicheskikh svoisty protoplazmy rastitel'nykh kletok. I. izmeneniya pronitsaemosti i vyazkosti plazmy v kletkakh cheshui luka i konchikov kapusty (Change of Colloid-Chemical Properties of the Protoplasm of Plant Cells with Age. I. Changes in Permeability and Viscosity of the Protoplasm in Cells of the Scale Skin of Onion and the Tips of Cabbage). –Doklady Akademii Nauk SSSR, 42 (5): 236. 1944a.

170. MAKSIMOV, N. A., and L. V. MOZHAEVA. Vozrastnye izmeneniya kolloidno-khimicheskikh svoisty protoplazmy rastitel'nykh kletok. II. Izmeneniya pronitsaemosti i vyazkosti plazmy v kletkakh list'ev konskikh bobov i ovsa (Changes of the Colloidal-Chemical Properties of the Protoplasm of Plant Cells with Age. II. Changes in Permeability and Viscosity of the Protoplasm of the Cells of Horse Bean Leaves and Oats). –Doklady Akademii Nauk SSSR, 42 (6): 291. 1944b.

171. MESHCHERSKAYA, K. A. Osobennosti reaktsii polovykh kletok na deistvie agentov v subletal'nykh dozakh (Specific Properties of the Reaction of Sex Cells to the Action of Sublethal Doses of Various Agents). –Arkhiv Biologicheskikh Nauk, 37 (3): 827. 1935.

172. MESHCHERSKAYA, K. A. Issledovanie yadov aminovykh sistem i ikh obezvrezhivaniya na yadernykh eritrotsitakh (Study the Affect on Nucleated Erythrocytes of Amino-System Poisons and their Detoxication).–Farmakologiya i Toksikologiya, 2 (3): 44. 1939.

173. MIKHAILOV, A. N. Kolloidnaya khimiya tannidov (Colloidal Chemistry of Tannins), pp. 1–320. Moskva-Leningrad. 1935.

174. MIKHEL'SON, N. I. Vliyanie anaerobioza i otravleniya monoiodatsetatnoi kislotoi na toki pokoya skeletnoi myshtsy (Effect of Anaerobiosis and Monoiodoacetic Acid Poisoning on Resting Currents in Skeletal Muscle). –Fiziologicheskii Zhurnal SSSR, 19 (5): 987. 1935.

175. MIROPOL'SKII, S. V. O mekhanizme mezhorgannogo belkovogo obmena (Mechanism of Interstitial Protein Metabolism). –Trudy Leningradskogo Obshchestva Estestvoispytatelei, 69 (5): 122. 1950.

176. MISHENEVA, V. S. K biokhimicheskoi kharakteristike sostoyaniya parabioza v nerve (Biochemical Characterization of Parabiotic Condition of the Nerve). Thesis. –Leningrad, Institut Fiziologii Akademii Nauk SSSR, 1955.

177. MOZHAEVA, L.V. Deistvie poverkhnostno-aktivnykh veshchestv na postuplenie vody v rastitel'nuyu kletku (Effect of Surface-Active Substances on the Entrance of Water into the Plant Cell). – Doklady Akademii Nauk SSSR 57(5): 509. 1947.

178. MOZHAEVA, L.V. Vliyanie temperatury i aeratsii na postuplenie vody v rastushchie i zakanchivayushchie rost kletki (Effect of Temperature and Aeration on the Entrance of Water into Growing and Mature Cells). – Doklady Akademii Nauk SSSR 72(1): 193. 1950a.

179. MOZHAEVA, L.V. Vliyanie solei i sakharozy na postuplenie vody v molodye rastitel'nye kletki (Effect of Salts and Sucrose on the Entrance of Water into Young Plant Cells). – Doklady Akademii Nauk SSSR, 72(6): 1167. 1950b.

180. MOZHAEVA, G.N. Zavisimost' velichiny mestnoi elektricheskoi reaktsii ot sily razdrazheniya v normal'nom i al'terirovannom nerve lyagushki (Dependence of the Magnitude of Local Electric Reaction on the Intensity of Excitation in a Normal and Altered Frog Nerve). – Tezisy Dokladov Nauchnoi Konferentsii Instituta Tsitologii Akademii Nauk SSSR, Posvyashchennoi 40-i, Godovshchine Velikoi Oktyabr'skoi Sotsialisticheskoi Revolyutsii, p. 18, Leningrad. 1958a.

181. MOZHAEVA, G.N. K voprosu o metodakh otvedeniya mestnoi elektricheskoi reaktsii nerva (On Methods of Conduction of the Local Electric Reaction of the Nerve). – Biofizika, 3(1): 31. 1958b.

182. MOZHAEVA, G.N. Vliyanie sily razdrazheniya na velichinu mestnoi elektricheskoi reaktsii nerva (Effect of Intensity of Excitation on the Magnitude of Local Electric Reaction of the Nerve). – Biofizika, 3(3): 286. 1958c.

183. MOZZHUKHIN, A.S. K voprosu o roli uglevodnogo obmena v podderzhanii toka povrezhdeniya (The Role of Carbohydrate Metabolism in the Maintenance of the Injury Current). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 26(6): 412. 1948.

184. MOZZHUKHIN, A.S. O vzaimootnoshenii mezhdu rasprostraneniem povrezhdeniya i potentsialom povrezhdeniya (The Interrelationship between Spread of Damage and the Injury Potential). – Trudy Vsesoyuznoi Meditsinskoi Akademii im Kirova, Vol. 45: 27. 1950a.

185. MOZZHUKHIN, A.S. O vzaimootnoshenii mezhdu potentsialom vozbuzhdeniya i potentsialom povrezhdeniya (The Interrelationship between Excitation Potential and Injury Potential). – Trudy Vsesoyuznoi Meditsinskoi Akademii im Kirova, Vol. 45: 19. 1950b.

186. MOZZHUKHIN. A.S. Rol' obmena veshchestv v proiskhozhdenii bioelektricheskikh yavlenii v skeletnoi myshtse (Role of Metabolism in the Generation of Bioelectric Phenomena in Skeletal Muscle). – Author's Summary, pp. 1-16. Leningrad. 1953.

187. MUZHEEV, V., and S. BORZDYKO. Izmenenie toka pokoya skeletnoi myshtsy lyagushki v zavisimosti ot razlichnykh nagruzok (Change in Resting Current of a Skeletal Frog Muscle as a Function of Different Loads). – Biologicheskii Zhurnal, 2(1): 70. 1933.

188. NASONOV, D.N. Vliyanie neelektrolitov na soderzhanie vody v zhivykh myshtsakh i nabukhayushchei zhelatine (Effect of Nonelectrolytes on Water Content of Live Muscles and Swelling Gelatine). – Arkhiv Anatomii, Gistologii i Embriologii, 19(1): 116. 1938.

189. NASONOV, D.N. Kontraktury poperechnopolosatykh myshts, poluchennye deistviem izoamilovogo spirta (Contractions of Striated Muscles Caused by the Action of Isoamyl Alcohol). – Doklady Akademii Nauk SSSR, 63(5): 597. 1948a.

190. NASONOV, D.N. Mestnaya reaktsiya, zakon "vse ili nichego" i avtomaticheskaya deyatel'nost' (Local Reaction, Law of "All or None" and Automatic Activity). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 4: 381. 1948b.

191. NASONOV, D.N. Kontraktury poperechnopolosatykh myshts, vyzvannye deistviem sulemy (Contractions of Striated Muscles Caused by Mercury Chloride). – Doklady Akademii Nauk, SSSR, 64(4): 595. 1949a.

192. NASONOV, D.N. Rol' energii razdrazhayushchego toka pri vozniknovenii vozbuzhdeniya v nervnom volokne (The Role of Energy of Excitatory Current in the Appearance of Excitation in the Nerve Fiber). – Fiziologicheskii Zhurnal SSSR, 41(4): 554. 1955.

193. NASONOV, D. and M. AVERBAKH. K voprosu o prirode avtomaticheskoi aktivnosti provodyashchikh tkanei (On the Nature of Automatic Activity of Conducting Tissues). – Zhurnal Obshchei Biologii, 12(4): 266. 1951.

194. NASONOV, D., M. AVERBAKH, and G. KOMAROVA. Avtomaticheskaya aktivnost' odinochnykh nervnykh volokon kraba Hyas araneus (Automatic Activity of Single Nerve Fibers of the Crab Hyas araneus). – Doklady Akademii Nauk SSSR, 73(4): 853. 1950.

195. NASONOV, D.N. and E.I. AIZENBERG. Vliyanie neelektrolitov na soderzhanie vody v zhivykh i ubitykh myshtsakh (Effect of Nonelectrolytes on the Water Content of Living and Killed Muscles). – Biologicheskii Zhurnal, 6(1): 165. 1937.

196. NASONOV, D.N. and V.Ya. ALEKSANDROV. K voprosu ob izmeneniyakh zhivogo veshchestva pri obratimom perekhode ego v mertvoe sostoyanie (Changes Taking Place in Living Matter During its Transition to Death). – Arkhiv Biologicheskikh Nauk, 36 (1): 95. 1934.
197. NASONOV, D.N. and V.Ya ALEKSANDROV. O mekhanizme toksicheskogo deistviya veshchestv na protoplazmu (The Mechanism of Toxic Effect of Substances on the Protoplasm). – Biologicheskii Zhurnal, 6 (1): 117. 1937.
198. NASONOV, D.N. and V.Ya ALEKSANDROV. Reaktsiya zhivogo veshchestva na vneshnie vozdeistviya (Reaction of Living Matter to External Influences), pp. 1-252. – Moskva-Leningrad. 1940.
199. NASONOV, D.N. and V.Ya. ALEKSANDROV. Printsip diffuzii i raspredeleniya v probleme kletochnoi pronitsaemosti (Principle of Diffusion and Distribution in the Problem of Cell Permeability). – Uspekhi Sovremennoi Biologii, 16 (6): 577. 1943a.
200. NASONOV, D.N. and V.Ya. ALEKSANDROV. O prichinakh vozniknoveniya bioelektricheskikh potentsialov (Causes for Appearance of Bioelectric Potentials). – Uspekhi Sovremennoi Biologii, 17 (1): 1. 1944.
201. NASONOV, D.N. and V.Ya. ALEKSANDROV. Vliyanie vozobnovleniya poperechnogo razreza na tok povrezhdeniya skeletnykh myshts. (K krittke membrannoi teorii bioelektricheskikh tokov) (Effect of Regeneration of a Nerve Cross Section on the Current of Damage of Skeletal Muscles. (A critical Appraisal of the Membrane Theory of Bioelectric Currents)). – Fiziologicheskii Zhurnal SSSR, 36 (6): 666. 1950.
202. NASONOV, D.N. and K.S. RAVDONIK. Reaktsiya izolirovannykh poperechnopolosatykh myshts lyagushki na slyshimye zvuki (Reaction of Isolated Striated Frog Muscles to Audible Sounds). – Fiziologicheskii Zhurnal SSSR, 33 (5): 569. 1947.
203. NASONOV, D.N. and K.S. RAVDONIK. Pryamoe vliyanie slyshimykh zvukov na nervnye kletki izolirovannykh spinno-mozgovykh gangliev krolika (Direct Effect of Audible Sounds on Nerve Cells of Isolated Spinal Ganglia of the Rabbit). – Doklady Akademii Nauk SSSR, 71 (5): 985. 1950.
204. NASONOV, D.N. and D.L. ROZENTAL'. Ob elektricheskom mekhanizme rasprostraneniya povrezhdeniya v poperechnopolosatykh myshechnykh voloknakh (The Electric Mechanism of Spread of Damage in Striated Muscle Fibers). – Zhurnal Obshchei Biologii, 8 (4): 281. 1947.
205. NASONOV, D.N. and D.L. ROZENTAL'. Kontraktury poperechnopolosatykh myshts, vyzvannye solyanokislym khininom (Contractions of Striated Muscles Caused by Quinine Hydrochloride). – Doklady Akademii Nauk SSSR, 63 (6): 765. 1948.
206. NASONOV, D.N. and D.L. ROZENTAL'. Pryamoe vliyanie slyshimykh zvukov na epitelii pochechnykh kanal'tsev lyagushki (Direct Effect of Audible Sounds on the Epithelium of Renal Tubules of the Frog). – Doklady Akademii Nauk SSSR, 71 (6): 1163. 1950.
207. NASONOV, D.N. and D.L. ROZENTAL'. Protiv tak nazyvaemogo zakona "Vse ili nichego" v fiziologii (Against the So-Called "All or None" Law in Physiology). – Uspekhi Sovremennoi Biologii, 34 (2): 161. 1952.
208. NASONOV, D.N. and D.L. ROZENTAL'. Faktor vremeni pri otsenke vozbudimosti tkanei (The Time Factor in Evaluation of Tissue Excitability). – Fiziologicheskii Zhurnal SSSR, 39 (4): 405. 1953.
209. NASONOV, D.N. and D.L. ROZENTAL'. Eshche ob izmerenii vozbudimosti provodyashchikh tkanei (Additional Data on the Measurement of Excitability of Conducting Tissues). – Fiziologicheskii Zhurnal SSSR, 41 (1): 121. 1955.
210. NASONOV, D.N. and D.L. ROZENTAL'. Izmenenie vozbudimosti nerva pri ego otdelenii ot tsentrov (Change in Nerve Excitability upon Separation of the Nerve from the Centers). – Fiziologicheskii Zhurnal SSSR, 42 (1): 78. 1956.
211. NASONOV, D.N. and T.A. SPERANSKAYA. O bioelektricheskikh potentsialakh v tkanyakh, zafiksirovannykh formalinom (Bioelectric Potentials of Tissues Fixed in Formalin). – In: Sbornik "Pamyati Akademika A.A. Zavarzina" p. 516. Moskva-Leningrad. 1948.
212. NASONOV, D.N. and I.P. SUZDAL'SKAYA. Stoikoe vozbuzhdenie, povrezhdenie i narkoz poperechnopolosatykh myshts. Soobshchenie I. Kontraktury ot etilovogo spirta, efira, NaCl KCl i HCl (Stable Excitation Damage and Narcosis of Striated Muscles. Communication 1. Contractions Caused by Ethyl Alcohol, Ether, NaCl, KCl and HCl). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 4: 393. 1948.
213. NASONOV, D.N. and I.P. SUZDAL'SKAYA. Gistofiziologicheskie issledovaniya deyatel'nosti podzheludochnoi zhelezy (Histophysiological Investigation of Pancreatic Activity). – Arkhiv Anatomii, Gistologii i Embriologii, 30 (4): 32. 1953.

214. NASONOV, D.N. and I.P. SUZDAL'SKAYA. O sootnoshenii mezhdu vozbudimost'yu i ritmicheskoi aktivnost'yu nervnoi tkani (The Relationship between Excitability and Rhythmic Activity of Nerve Tissue). – Uchenye Zapiski Leningradskogo Universiteta, Vol. 164: 286. 1954.

215. NASONOV, D.N. and I.P. SUZDAL'SKAYA. Vliyanie temperatury na vozbudimost' nerva lyagushki (Effect of Temperature on Excitability of the Frog Nerve). – Fiziologicheskii Zhurnal SSSR, 42 (4): 415. 1956a.

216. NASONOV, D.N. and I.P. SUZDAL'SKAYA. Vliyanie temperatury na vozbudimost' nerva krysy (Effect of Temperature on Excitability of the Rat Nerve). – Fiziologicheskii Zhurnal SSSR, 42 (6): 464. 1956b.

217. NASONOV, D.N. and I.P. SUZDAL'SKAYA. Vliyanie temperatury na vozbudimost' periphericheskikh nervov khladnokrovnykh i teplokrovnykh zhivotnykh (reptilii, ptitsy) (Effect of Temperature on Excitability of Peripheral Nerves of Cold Blooded and Warm-Blooded Animals (reptiles, birds)). – Biofizika, 1 (4): 305. 1956c.

218. NASONOV, D.N. and I.P. SUZDAL'SKAYA. K voprosu ob izmeneniyakh, proiskhodyashchikh v protoplazme myakotnykh nervnykh volokon pri vozbuzhdenii (Changes in the Protoplasm of Medullated Nerve Fibers upon Stimulation). – Fiziologicheskii Zhurnal SSSR, 43 (7): 664. 1957.

219. NASONOV, D.N. and I.P. SUZDAL'SKAYA. Vliyanie povysheniya temperatury na vozbudimost' nerva krysy (Effect of Temperature Rise on Excitability of the Rat Nerve). – Fiziologicheskii Zhurnal SSSR, 44 (11): 1034. 1958.

220. NAVAKATIKYAN, A.O. Uravnenie Nasonova-Rozental' i razlichnye pokazateli vozbudimosti (The Nasonov-Rozental' Equation and Various Indexes of Excitability). – Fiziologicheskii Zhurnal SSSR, 40 (6): 756. 1954.

221. OPARIN, A.I. Vozniknovenie zhizni na zemle (Origins of Life on Earth). pp. 1-268. – Moskva-Leningrad. 1941.

222. OPARIN, A.I. Belok kak osnova zhiznennykh protsessov (Protein as the Basis of Life Processes). – In: Sbornik "Soveshchanie po belku", p. 5. – Moskva-Leningrad. 1948.

223. OPARIN, A.I. and V.V. YURKEVICH. Ob adsorbtsii fermentov drozhzhevymi kletkami (Absorption of Enzymes by Yeast Cells). – Doklady Akademii Nauk SSSR, 66 (2): 247. 1949.

224. PANTELEEVA, N.S. Osobennosti obmena fosfornykh soedinenii pri tetanuse, tonuse i knotrakturakh (Characteristics of Metabolism of Phosphorus Compounds in Tetanic and Tonic Contractions). – Vestnik Leningradskogo Universiteta, Vol. 10: 115. 1953.

225. PASYNSKII, A.G. O Prirode strukturnykh prevrashchenii belkovykh veshchestv. Belki, ikh spetsificheskie svoistva (On the Nature of Structural Transformations of Proteins. Proteins, their Specific Properties), pp. 1-144. – Kiev. 1955.

226. PASYNSKII, A.G., M.S. VOLKOVA, and V.P. BLOKHINA. Izotopnaya metodika opredeleniya denaturatsionnykh izmenenii v belkakh (The Isotope Method of Determining Denaturation Changes in Proteins). – Doklady Akademii Nauk SSSR, 101 (2): 317. 1955.

227. PAVLOVSKAYA, T.E., M.S. VOLKOVA and A.G. PASYNSKII. Izmenenie svyazyvaniya metionina S^{35} syvorotkoi krovi pri denaturatsii oblucheniem i nagrevaniem (Change in Binding of S^{35} Methionine by Blood Serum upon Denaturation by Irradiation and by Heating). – Doklady Akademii Nauk SSSR, 10 (4): 723. 1955.

228. RAEVSKAYA, M.A. Vozniknovenie i rasprostranenie povrezhdenii v poperechnopolosatykh myshtsakh lyagushki pod vliyaniem mekhanicheskikh vozdeistvii (Appearance and Spread of Damage in Striated Frog Muscles under the Influence of Mechanical Agents). – In: Sbornik "Pamyati akademika A.A. Zavarzina", p. 443. Moskva-Leningrad. 1948.

229. RAEVSKAYA, M.A. and A.S. TROSHIN. O mekhanizme narkoticheskogo deistviya nekotorykh veshchestv na spermatozoidy Rana temporaria (Mechanism of Narcotic Effect of Certain Substances on Spermatozoa of Rana temporaria). – Biologicheskii Zhurnal, 6 (4): 721. 1937.

230. RAVDONIK, K.S. K voprosu o pryamom deistvii slyshimykh zvukov na izolirovannye poperechnopolosatye myshtsy (Direct Effect of Audible Sounds on Isolated Striated Muscles). – Doklady Akademii Nauk SSSR, 66 (2): 293. 1949.

231. REZVYAKOV, N.P. Izmeneniya v funktsional'nykh svoistvakh nerva pod vliyaniem shchelochnykh solei margantsevoi kisloty (Changes in Functional Properties of the Nerve under the Influence of Basic Salts of Permanganic Acid). – Rabochaya Fiziologicheskaya Laboratoriya Petrogradskogo Universiteta, Vol. 9-10: 53. 1914-1915.

232. REZVYAKOV, N.P. K voprosu o dekremente volny vozbuzhdeniya pri parabioze (On the Decrement in the Excitation Wave in Parabiosis). – Fiziologicheskii Zhurnal SSSR, 17 (2): 336. 1934.

233. ROMANOV, S.N. Svyazyvanie krasitelya nervnymi kletkami v razlichnye sroki posle razdrazheniya (Binding of Dye by Nerve Cells at Varying Time Periods after Excitation). – Doklady Akademii Nauk SSSR, 61(5): 909. 1948a.

234. ROMANOV, S.N. Vliyanie prodolzhitel'nosti razdrazheniya nervnykh kletok na vlichinu svyazyvaniya imi krasitelei (Effect of Duration of Excitation of Nerve Cells on the Extent of their Binding of Dyes). – Doklady Akademii Nauk SSSR, 61(4): 761. 1948b.

235. ROMANOV, S.N. Vliyanie predvaritel'nogo nagrevaniya myshts na velichinu svyazyvaniya imi krasitelya (Effect of Prior Heating of Muscles on their Affinity for Various Dyes). – Doklady Akademii Nauk SSSR, 66(2): 297. 1949a.

236. ROMANOV, S.N. Vliyanie razlichnykh kontsentratsii spirta na sorbtsionnye svoistva kletok simpaticheskoi i animal'noi nervnoi sistemy (Effect of Various Concentrations of Alcohol on the Sorptional Properties of Cells of the Sympathetic and Animal Nervous Systems). – Doklady Akademii Nauk SSSR, 69(3): 473. 1949b.

237. ROMANOV, S.N. Izmeneniya sorbtsionnykh svoistv nervnykh kletok krolika pri travmaticheskom shoke (Changes in the Sorptional Properties of Rabbit Nerve Cells in Traumatic Shock). – Zhurnal Obshchei Biologii, 10(2): 76. 1949c.

238. ROMANOV, S.N. Vliyanie uslovii vyrashchivaniya drozhzhei na ikh sorbtsionnye svoistva (Effect of Growth Conditions on the Sorptional Properties of Yeasts). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 32(9): 224. 1951.

239. ROMANOV, S.N. Zavisimost' mezhdu sorbtsionnymi svoistvami golovnogo mozga myshei i soprotivlyaem t'yu organizma deistviyu strikhnina (Relationship between Sorptional Properties of the Mouse Brain and the Resistance of the Organism to Strychnine).– Doklady Akademii Nauk SSSR, 89(4): 753. 1953a.

240. ROMANOV, S.N. Izmeneniya sorbtsionnykh svoistv nervnykh kletok golovnogo mozga pod vliyaniem uslovno-reflektornogo razdrazhitelya (Changes in the Sorptional Properties of Brain Nerve Cells under the Influence of a Conditioned Stimulus). – Doklady Akademii Nauk SSSR, 90(1): 117. 1953b.

241. ROMANOV, S.N. Reaktsiya kletok organizma na zvuki ot vzryva (Reaction of the Cells of the Organism to Sounds of Explosion). – Fiziologicheskii Zhurnal SSSR, 40(1): 86. 1954.

242. ROZENTAL', D.L. O prichinakh rasprostraneniya povrezhdeniya y poperechnopolosatykh myshchenykh voloknakh (The Causes of Spread of Damage in Striated Muscle Fibers). Thesis. – Institut Experimental'noi Meditsiny Akademii Meditsinskikh Nauk SSSR. Leningrad. 1946.

243. ROZENTAL' D.L. Kontraktury poperechnopolosatykh myshts, vyzvannye $CaCl_2$, $MgCl_2$, $BaCl_2$ (Contractions of Striated Muscles Caused by $CaCl_2$, $MgCl_2$, $BaCl_2$). – Doklady Akademii Nauk SSSR, 63(5): 593. 1948.

244. ROZENTAL' D.L. Izmenenie okrashivaemosti gangliev krysy pri razdrazhenii nerva impul'sami razlichnoi chastoty (Change in Staining Properties of Rat Ganglia Upon Excitation of the Nerve by Impulses of Varying Frequencies). – Manuscript. Institut Tsitologii Akademii Nauk SSSR. 1958.

245. ROZENTAL', D.L. and V.N. FILIPPOVA. K voprosu ob opredelenii vozbudimosti myshts cheloveka (Determination of Excitability of Human Muscles). – Byulleten' Eksperimental'noi Biologii i meditsiny, 43(1): Appendix 132. 1957.

246. ROZENTAL', D.L. and E.A. SHAPIRO. Vozbudimost' nerva kraba Hyas coarctatus pri izmenenii mezhelektrodnogo rasstoyaniya (Excitability of the Nerve of the Crab Hyas coarctatus with Changes in Interelectrode Distance). – Doklady Akademii Nauk SSSR, 108(5): 977. 1956.

247. RUBIN, L.R. K voprosu o kondensatornom metode khronaksimetrii (The Condenser Method of Chronaximetry). – Zhurnal Nevropatologii i Psikhiatrii, 8(6): 102. 1939.

248. RUBIN, L.R. and E.A. FEDOROVA. O klinicheskoi khronaksimetrii (Clinical Chronaximetry). – Zhurnal Nevropatologii i Psikhiatrii, 20(4): 59. 1951.

249. RUBINSHTEIN, D.L. Sushchestvuet li polupronitsaemaya kletochnaya obolochka ? (Does a Semi-permeable Cell Membrane Exist ?). – In: Sbornik "Problema pronitsaemosti", pp. 7, 72, 112, 201. Moskva, Trudy Konferentsii Moskovskogo Obshchestva fiziologov. 1939.

250. RUBINSHTEIN, D.L. Obshchaya fiziologiya (General Physiology), pp. 1-648. – Moskva. 1947.

251. RUBINSHTEIN, D.L. Vystuplenie po dokladu D.N. Nasonova na Konferentsii v Gagrakh v 1947 (Discussion of D.N. Nasonov's paper at the Conference at Gagry, 1947). Gagry Symposium, p. 34. – Tbilisi. 1949a.

252. RUBINSHTEIN, D.L. Krizis membrannoi teorii bioelektricheskikh potentsialov (Crisis of the Membrane Theory of Bioelectric Potentials). – Gagry Symposium, 1, p. 85. – Tbilisi. 1949b.

253. RUBINSHTEIN, D.L. and I. VERKHOVSKAYA. Ob"emnyi metod izucheniya pronitsaemosti drozhzhevykh kletok (The Volumetric Method for the Study of Permeability of Yeast Cells). – Mikrobiologiya, 4(3): 342. 1935.

254. RUSINOV, V.S. O stoikoi mestnoi volne myshechnogo vozbuzhdeniya (The Stable Local Wave of Muscle Excitation). – Trudy Fiziologicheskogo Instituta Leningradskogo Universiteta, Vol. 17: 10. 1936.

255. SAICHUK, V.I. Raspredelenie amino-azota mezhdu eritrotsitami i plazmoi krovi loshadei v norme i pri infektsionnoi anamii (Normal Distribution of Amino-Nitrogen between Erythrocytes and Blood Plasma in Horses and Alterations in It in Infectious Anemia). –Thesis. – Sverdlovsk-Moskva. 1941.

256. SANT-ILER [St. Hilaire], K.K. Dvoyakoe deistvie vysykhayushchego nerva na myshtsy (The Double Effect of a Drying Nerve on the Muscle). – Zapiski imperatozskoi Akademii Nauk, Vol. 61: 73. 1890.

257. SECHENOV, I.M. Pererezka nerva, kak usloviye narastaniya ego razdrazhitel'nosti (Cutting of a Nerve as a Condition for Increasing its Excitability). – Meditsinskii Vestnik, Vol.33: 301; Vol. 34: 313 1861.

258. SHAPIRO, E.A. Izmenenie sorbtsionnykh svoistv okonchanii i kontsevykh razvetvlenii dvigatel'nogo nerva pozvonochnykh v usloviyakh fiziologicheskogo vozbuzhdeniya (Change in the Sorptional Properties of the Endings and the End Branches of the Motor Nerve of Vertebrates under Condition of Physiological Stimulation). – Doklady Akademii Nauk SSSR, 88(6): 1075. 1953.

259. SHAPIRO, E.A. Sorbtsionnye svoistva myshts pri tetanicheskom sokrashchenii (Sorptional Properties of Muscles during Tetanic Contraction). – Manuscript. Institut Tsitologii Akademii Nauk SSSR. 1958.

260. SHTUTINA, B.L. Obratimost' granuloobrazovaniya pri paranekroze (Resersibility of Granule Formation in Paranecrosis). – Arkhiv Anatomii, Gistologii i Embriologii, 22(1): 74. 1939.

261. SHCHERBAKOV, B.I. and N.L. SEMIOTROCHEVA. Pogloshchenie vody zhivymi rastitel'nymi kletkami kak aktivnyi fiziologicheskii protsess (Absorption of Water by Living Plant Cells as an Active Physiological Process). – Doklady Akademii Nauk SSSR, 93(4): 721. 1953.

262. SISAKYAN, N.M. Fermentativnaya aktivnost' protoplazmennykh struktur (Enzymatic Activity of Protoplasmic Structures), pp. 1-92. – Moskva. 1951.

263. SMITTEN, N.A. Vital'naya okraska neironov lyagushki pri razdrazhenii (Vital Staining of Frog Neurons Upon Excitation). – In: Sbornik "Pamyati Akademika A.A. Zavarzina", pp. 482. Moskva-Leningrad. 1949.

264. SMOILOVSKAYA, E. O supravital'nom okrashivanii perezhivayushchikh srezov normal'nykh tkanei v fiziologicheskoi i gipotonicheskoi sredakh (Supravital Staining of Surviving Slices of Normal Tissues in Physiological and Hypotonic Media). – Arkhiv Anatomii, Gistologii i Embriologii, 19(1-2): 105. 1938.

265. SORENI, E.T. and O.L. CHEPINOGA. O novom spetsificheskom svoistve miozina (A New Specific Property of Myosin). – Ukrainskii Biokhimicheskii Zhurnal, 18(2): 159. 1946.

266. STEPANOVA, S.S. and E.M. KROKHINA. Opyt izucheniya zhivykh nervnykh kletok i sinapsov v temnopol'nom kondensore (A Study of Living Nerve Cells and Synapses in a Dark Field Condenser). – Arkhiv Biologicheskikh Nauk, 61(2): 107. 1941.

267. SUBBOTIN, M. Ob izmenenii nervnoi razdrazhitel'nosti pri deistvii na nervy razdrazhitelei khimicheskikh (Change in Nerve Excitability under the Influence of Chemical Irritants). – Sovremennaya Meditsinskaya Gazeta dlya Vrachei, p. 524, Kiev. 1866.

268. SUZDAL'SKAYA, I.P. O narkoze kletochnykh elementov vegetativnoi nervnoi sistemy (Narcosis of Cellular Elements of the Autonomic Nervous System). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 4: 411. 1948a.

269. SUZDAL'SKAYA, I.P. Kontraktury poperechnopolosatoi muskulatury poluchennye deistviem NaOH (Contractions of Striated Muscles Caused by NaOH). – Doklady Akademii Nauk SSSR, 63(6): 769. 1948b.

270. SUZDAL'SKAYA, I.P. O mekhanizme fiziologicheskogo deistviya solei na poperechnopolosatye myshtsy (The Mechanism of Physiological Effect of Salts on Striated Muscles). – Vestnik Leningradskogo Universiteta, Vol. 7: 37. 1952.

271. SUZDAL'SKAYA, I.P. Reaktsiya pishchevaritel'nykh zheley myshtsy na gidrostaticheskoe davlenie (The Reaction of the Digestive Glands of Muscle [sic.] to Hydrostatic Pressure). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 39(3): 22. 1955.

272. SUZDAL'SKAYA, I.P. Vliyanie temperatury na vozbudimost' myshts lyagushki (Effect of Temperature on Excitability of Frog Muscles). – Fiziologicheskii Zhurnal SSSR, 43(1): 80. 1957a.

273. SUZDAL'SKAYA, I.P. Vliyanie temperatury na vozbudimost' myshts krysy (Effect of Temperature on Excitability of Rat Muscles). – Fiziologicheskii Zhurnal SSSR, 43(5): 449. 1957b.

274. SZENT-GYORGYI, A. O myshechnoi deyatel'nosti (Muscular Activity), pp. 1-176. – Moskva. 1947.
275. TARUSOV, B.N. Polyarizatsionnye svoistva myshtsy pri obrabotke formalinom (Properties of Polarization of Muscle Treatment with Formalin). – I sessiya Moskovskogo Obshchestva Fiziologov, Biokhimikov i Farmakologov, Sbornik Dokladov, p. 230. Moskva-Leningrad. 1941.
276. TARUSOV, B. and V. BURLAKOVA. O sostoyanii kaliya vnutri eritrotsitov (State of Potassium Present in Erythrocytes). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 7(5): 393. 1939.
277. TONGUR, V.S. Biologicheskoe deistvie vysokikh davlenii (Biological Effect of High Pressure). – Uspekhi Sovremennoi Biologii, 23(3): 445. 1947.
278. TONGUR, V.S. Vliyanie temperatury i davleniya na rastvor yaichnogo belka (Effect of Temperature and Pressure on a Solution of Egg Proteins). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 26(6): 440. 1948.
279. TONGUR, V.S. Vliyanie davleniya na denaturatsiyu yaichnogo belka (Effect of Pressure on Denaturation of Egg Protein). – Kolloidnyi Zhurnal, Vol. 11: 274. 1949.
280. TONGUR, V.S. and N.A. KAZMINA. Vliyanie davleniya na belki. Denaturatsiya syvorotochnogo al'bumina pod davleniem (Effect of Pressure on Proteins. Denaturation of Serum Albumin under Pressure). – Biokhimiya, 15(3): 212. 1950.
281. TONGUR, V.S. and V.I. KASATOCHKIN. Obratimost' teplovoi denaturatsii belka pod davleniem (Reversibility of Heat Denaturation of Protein under Pressure). – Doklady Akademii Nauk SSSR, 74(3): 553. 1950.
282. TONGUR, V.S. and V.I. KASATOCHKIN. Vliyanie vysokikh davlenii na teplovuyu denaturatsiyu belka. Khimiya i fiziko-khimiya vysokomolekulyarnykh soedinenii (Effect of High Pressures on Heat Denaturation of Proteins. Physical Chemistry of High Molecular Compounds), pp. 1-124. – Moskva. 1952.
283. TONGUR, V.S. and A.M. TONGUR. Regeneratsiya insulina pod davleniem (Resynthesis of Insulin under Pressure). – Biokhimiya, 16(5): 410. 1951.
284. TRIFONOVA, A.N. K fiziologii dedifferentsirovki i rosta. Vliyanie asfiksii na razvitie i kariokineticheskoe delenie u embrionov ryb (Physiology of Dedifferentiation and Growth. Effect of Asphyxia on Development and Karyokinetic Division in Fish Embryos). – Arkhiv Biologicheskikh Nauk, 37(3): 757. 1935.
285. TROSHIN, A.S. O raspredelenii vody i khlora mezhdu drozhzhevymi kletkami i sredoi (Distribution of Water and Chlorine between Yeast Cells and the Medium). – Biokhimiya, 13(3): 253. 1948a.
286. TROSHIN, A.S. Solevye toki na kompleksnom koatservate: zhelatina i gummiarabik (Salt Currents in a Complex Coacervate: Gelatin and Gum-Arabic). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 4: 425. 1948b.
287. TROSHIN, A.S. Raspredelenie veshchestv mezhdu kletkoi i sredoi, Soobshchenie 1. Raspredelenie galaktozy i sakharozy v kompleksnykh koatservatnykh sistemakh. (Model'nye opyty) (Distribution of Substances between the Cell and the Medium. Communication 1. Distribution of Galactose and Sucrose in Complex Coacervate Systems (model experiments)). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 31(3): 180. 1951a.
288. TROSHIN, A.S. Raspredelenie veshchestv mezhdu kletkoi i sredoi. Soobshchenie 2. Raspredelenie galaktozy mezhdu eritrotsitami krolika i okruzhayushchei ravnovesnoi zhidkost'yu (Distribution of Substances between the Cell and the Medium. Communication 2. Distribution of Galactose between Rabbit Erythrocytes and the Surrounding Liquid at Equilibrium). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 31(4): 285. 1951b.
289. TROSHIN, A.S. Raspredelenie vital'nykh krasitelei mezhdu myshtsami lyagushki i okruzhayushchim rastvorom (Distribution of Vital Stains between Frog Muscles and the Surrounding Fluid Medium). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 32(8): 162. 1951c.
290. TROSHIN, A.S. Raspredelenie alanina mezhdu myshtsami lyagushki i okruzhayushchei ravnovesnoi zhidkost'yu (Distribution of Alanine between Frog Muscles and the Surrounding Liquid at Equilibrium). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 33(9): 228. 1951d.
291. TROSHIN, A.S. O raspredelenii sakharov mezhdu kletkami i okruzhayushchei ikh sredoi (Distribution of Sugar between the Cells and Surrounding Medium). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 34(1): 59. 1951e.
292. TROSHIN, A.S. Raspredelenie kreatinina mezhdu myshtsami lyagushki i okruzhayushchei ikh sredoi (Distribution of Creatinine between Frog Muscles and the Surrounding Medium). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 34(1): 59. 1952.
293. TROSHIN, A.S. O regulyatsii soderzhaniya vody v protoplazme (Regualtion of the Water Content of the Protoplasm). – Trudy Zoologicheskogo Instituta Akademii Nauk SSSR, Vol. 13: 420. 1953.

294. TROSHIN, A.S. Raspredelenie mocheviny mezhdu ikronozhnymi myshtsami lyagushki i sredoi (Distribution of Urea between the Gastrocnemius Muscles of the Frog and the Medium). – Biokhimiya, 19 (4): 400. 1954.

295. TROSHIN, A.S. Problema kletochnoi pronitsaemosti (The Problem of Cellular Permeability), pp. 1-474, Moskva-Leningrad, 1956.

296. TROSHIN, A.S. O svyazannom i svobodnom natrii v skeletnykh myshtsakh lyagushki (Bound and Free Sodium in Skeletal Muscles of the Frog). – Biofizika, 2 (5): 617. 1957.

297. TROSHIN, A.S. and L.N. PISAREVA. O svobodnom i svyazannom natrii i kalii v skeletnykh myshtsakh lyagushki (Free and Bound Sodium and Potassium in Skeletal Muscles of the Frog). – Tezisy Dokladov Nauchnoi Konferentsii Instituta Tsitologii Akademii Nauk SSSR, Posvyashcheannoi 40-i Godovshchine Velikoi Oktyabr'skoi Sotsialisticheskoi Revolyutsii, p. 28. Leningrad. 1958.

298. YAMPOL'SKAYA, M.N. Raspredelenie aminnogo i polipeptidnogo azota mezhdu eritrotsitami i plazmoi krovi (Distribution of Amine - and Polypeptide-Nitrogen Between Erythrocytes and Blood Plasma). – Fiziologicheskii Zhurnal SSSR, 24 (6): 942. 1938.

299. UFLYAND, Yu. M. Teoriya i praktika khronaksimetrii (Theory and Practice of Chronaximetry). pp. 1-298. – Leningrad. 1938.

300. UFLYAND, Yu. M. O znachenii issledovaniya khronaksii (Po povodu stat'i D.N. Nasonova i D.L. Rozental' "Faktor vremeni pri otsenke vozbudimosti tkanei") (The Importance of Studies of Chronaxy. (Reference to the Paper by D.N. Nasonov and D.L. Rozental' "The Time Factor in the Evaluation of Tissue Excitability")). – Fiziologicheskii Zhurnal SSSR, Vol. 40 (1): 106. 1954.

301. UKHTOMSKII, A.A. (1904) Ob izmeneniyakh razdrazhitel'nosti myshts pod vliyaniem utomleniya (Changes in Muscle Excitability under the Influence of Fatigue). Complete Works, Vol. 5: 5. – Leningrad. 1954.

302. UKHTOMSKII, A.A. (1926) Zakon "vse ili nichego" ("All or None Law"). Complete Works, Vol. 2: 31. – Leningrad. 1951.

303. UKHTOMSKII, A.A. (1927) Fiziologiya dvigatel'nogo apparata (Physiology of the Motor Apparatus). Complete Works, Vol. 3: 1-165. – Leningrad. 1951.

304. UKHTOMSKII, A.A. (1930) O nekotorykh novykh chertakh parabioza (Some New Features of Parabiosis). Complete Works, Vol. 2: 35. – Leningrad. 1951.

305. UKHTOMSKII, A.A. (1937) Fiziologicheskii pokoi i labil'nost' kak biologicheskie faktory (Physiological Rest and Lability as Biological Factors). Complete Works, Vol. 2: 122. – Leningrad. 1951.

306. UKHTOMSKII, A.A. (1940) Nekotorye sblizheniya i perspektivy v uchenii o fiziologicheskom vozbuzhdenii (Some Approximations and Perspectives of the Theory of Physiological Excitation). – Complete Works, Vol. 2: 155. – Leningrad. 1951.

307. UKHTOMSKII, A.A. (1945) Ocherk fiziologii nervnoi sistemy (Outlines of the Physiology of the Nervous System). – Complete Works, Vol. 4: 1-220, Leningrad. 1954.

308. USHAKOV, B.P. Vliyanie razdrazheniya na vital'nuyu okrasku nervnykh kletok spinno-mozgovogo uzla lyagushki (Effect of Excitation on Vital Staining of Nerve Cells of the Spinal Ganglia of the Frog). – Uchenye Zapiski Leningradskogo Universiteta, Vol. 99: 114. 1949.

309. USHAKOV, B.P. Izmenenie sorbtsionnykh svoistv nerva kraba Hyas araneus pri vozbuzhdenii (Change in Sorptional Properties of the Nerve of the Crab Hyas araneus upon Excitation). – Doklady Akademii Nauk SSSR, 71 (1): 205. 1950.

310. USHAKOV, B.P. Razvitie parabioticheskogo bloka skeletnoi myshtsy, vyzvannogo khloristym kaliem, etilovym spirtom i khloralgidratom (Development of a Parabiotic Block of Skeletal Muscle Caused by Potassium Chloride, Ethyl Alcohol and Chloral Hydrate). – Fiziologicheskii Zhurnal SSSR, 38 (3): 297. 1952a.

311. USHAKOV, B.P. Sootnoshenie porogov parabioticheskogo bloka i kontraktury somaticheskogo myshechnogo volokna (The Relationship between Thresholds of the Parabiotic Block and Contraction of the Somatic Muscle Fiber). – Doklady Akademii Nauk SSSR, 85 (3): 673. 1952b.

312. USHAKOV, B.P. Zavisimost' toksichnosti fermentativnykh yadov ot ikh kontsentratsii (Dependence of Toxicity of Enzyme Poisons on their Concentration). – Vestnik Leningradskogo Universiteta, Vol. 4: 101. 1953a.

313. USHAKOV, B.P. Zavisimost' narkoticheskogo deistviya tiolovykh yadov ot ikh kontsentratsii (Dependence of the Narcotic Effect of Thiol Poisons on their Concentration). – Doklady Akademii Nauk SSSR, 92 (1): 193. 1953b.

314. USHAKOV, B.P. Teploustoichivost' muskulatury rakoobraznykh v svyazi s usloviyami sushchestvovaniya vida (Thermostability of the Musculature of Crustaceans in Connection with Life Conditions of the Species). – Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya, Vol. 5: 67. 1956.

315. USHAKOV, B.P., M.S. AVERBAKH, I.P. SUZDAL'SKAYA, V.P. TROSHINA and T.N. CHEREPANOVA. O parabioticheskoi prirode fiziologicheskogo elektrotona (The Parabiotic Nature of Physiological Electrotonus). – Fizioligicheskii Zhurnal SSSR, 39 (2): 218. 1953.

316. USHAKOV, B.P. and T.A. DZHAMUSOVA. Osobennosti parabioza somaticheskoi myshtsy lyagushki, vyzvannogo deistviem monoiodatsetata (Characteristics of the Parabiosis of the Somatic Muscle of the Frog Caused by Monoiodoacetate). – Doklady Akademii Nauk SSSR, 94 (3): 593. 1954.

317. USHAKOV, B.P. and S.A. KROLENKO. Sravnitel'noe izuchenie toksichnosti monoiodatsetata dlya muskulatury pozvonochnykh i bespozvonochnykh zhivotnykh (Comparative Study of the Toxicity of Monoiodoacetate for Muscles of Vertebrate and Invertebrate Animals). – Fiziologicheskii Zhurnal SSSR, 40 (2): 208. 1954.

318. USHAKOV, B.P. and T.N. CHEREPANOVA. Vliyanie anoda i katoda na parabioticheskii blok somaticheskoi muskulatury lyagushki (Effect of the Anode and Cathode on the Parabictic Block of Somatic Musculature of the Frog). – Byulleten' Eksperimental'noi Biologii i Meditsiny 34 (10): 15. 1952.

319. YUDENICH, N.A. K fiziologii poperechnogo razreza. II. Deistvie postoyannogo toka na poperechnyi razrez nerva (The Physiology of a Nerve Cross Section. II. Effect of Direct Current on a Cross Section of a Nerve). – Zhurnal Eksperimental'noi Biologii i Meditsiny, 9 (23): 542. 1928.

320. YURKEVICH, V.V. Vydelenie invertazy drozhzhevymi kletkami (Production of Invertase by Yeast Cells). – Doklady Akademii Nauk SSSR, 94 (2): 329. 1954.

321. VASIL'EV, L.L. O kolloidnykh svoistvakh nervnogo stvola (Colloid Properties of the Nerve Trunk). – Russkii Fiziologicheskii Zhurnal, 5 (4/6): 284. 1922.

322. VERESHCHAGIN, S.M. K voprosu o denaturatsionnykh izmeneniyakh myshechnykh belkov vo vremya tonicheskikh sokrashchenii (On Denaturation Changes in Muscle Proteins During Tonic Contractions). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 27 (4): 291. 1949.

323. VERIGO, B.F. K voprosu o deistvii na nerv gal'vanicheskogo toka preryvistogo i nepreryvnogo. (Popytka ob'yasneniya fiziologicheskikh yavlenii elektrotona) (Effect of Discontinuous and Continuous Galvanic Current on the Nerve (An Attempt to Elucidate the Physiological Phenomena of Electrotonus)), pp. 1-338. – St. Peterburg. 1888.

324. VLADIMIROV, G.E., I.A. PELISHCHENKO and A.P. URINSON. Khod obnovleniya fosforsoderzhashchikh veshchestv v chelovecheskikh eritrotsitakh (The Course of Turnover of Phosphorus Containing Substances in Human Erythrocytes). – Fiziologicheskii Zhurnal SSSR, 33 (3): 350. 1947.

325. VLADIMIROV, G.E., I.A. PELISHCHENKO, and A.P. URINSON. Khod obnovleniya fosfora lipoidov i belkov stromy chelovecheskikh eritrotsitov (Course of Turnover of Phosphorus of Lipids and Proteins in the Stroma of Human Erythrocytes). – Biokhimiya, 13 (5): 449. 1948.

326. VOL'FENZON, L.G. Paranekroticheskoe deistvie mestnykh anesteitikov na kletochnye elementy razlichnykh tkanei (Paranecrotic Effect of Local Anaesthetics on Cellular Elements of Various Tissues). – Zhurnal Obshchei Biologii, 15 (3): 220. 1954.

327. VOROB'EV, V.E. Opyt issledovaniya konservirovannoi v rastvore sakharozy chelovecheskoi krovi (A Study of Human Blood Preserved in a Sucrose Solution). – Fiziologicheskii Zhurnal SSSR, 24 (6): 708. 1939.

328. VUL, I.M. and A.L. KONIKOV. Analiz dinamiki khronaksii i reobazy nervno-myshechnogo pribora pri vyklyuchenii vliyanii tsentral'noi nervnoi systemy (Analysis of the Dynamics of Chronaxy and Rheobase of a Nerve-Muscle Preparation following Exclusion of Influences of the Central Nervous System). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 4 (4): 374. 1937.

329. VVEDENSKII, N.E. (1883) O telefonicheskikh yavleniyakh v myshtse pri iskusstvennom i estestvennom tetanuse (Telephonic Phenomena in the Muscle during Artificial and Natural tetanus). Complete Works, Vol. 1: 176. –Leningrad. 1951.

330. VVEDENSKII, N.E. (1901) Vozbuzhdenie, tormozhenie i narkoz (Stimulation, Inhibition and Narcosis). Complete Works, Vol. 4: 9. –Leningrad. 1953.

331. ZARAKOVSKII, G.M. and S.V. LEVIN. Vliyanie razlichnoi sily razdrazheniya simpaticheskikh i spinal'nykh gangliev na svyazyvanie imi prizhiznennykh krasitelei (Effect of Varying Intensities of Excitation of Sympathetic and Spinal Ganglia on their Binding of Vital Stains). – Fiziologicheskii Zhurnal SSSR, 39 (1): 81. 1953.

332. ZBARSKII, B.I. Adsorbtsiya eritrotsitami produktov raspada belka i difteriinogo toksina (Adsorption by Erythrocytes of Decomposition Products of Protein and Diphtheria Toxin). – Zhurnal Eksperimental'noi Biologii i Meditsiny, 1 (1): 121. 1925a.

333. ZBARSKII, B.I. Adsorbtsiya produktov raspada belka formennymi elementami krovi (Adsorption of Protein Decomposition Products by Blood Cells). – Russkii Fiziologicheskii Zhurnal, 8 (1/2): 261. 1925b.

334. ZBARSKII, B.I. and L.R. ZUBKOVA. Rol' eritrotsitov v obmene belkov. II Raspredelenie aminoazota mezhdu eritrotsitami i plazmoi in vivo (Role of Erythrocytes in Protein Metabolism. II. Distribution of Amino-Nitrogen between Erythrocytes and the Plasma in Vivo). – Fiziologicheskii Zhurnal SSSR, 17 (3): 442. 1934.

335. ZBARSKII, B.I. Rol'eritrotsitov v obmene belkov. III. Raspredelenie aminoazota mezhdu eritrotsitami i plazmoi in vivo (Role of Erythrocytes in Protein Metabolism. II. Distribution of Amino-Nitrogen between Erythrocytes and the Plasma in Vivo). – Arkhiv Biologicheskikh Nauk. 41 (2): 49. 1936.

336. ZELENKOVA, N.P. Kontraktury poperechnopolosatykh myshts, vyzvannye deistviem khloralgidrata (Contractions of Striated Muscles Caused by Chloral Hydrate). – Doklady Akademii Nauk, SSSR, 64 (4): 591. 1949.

337. ZHINKIN, L.N. and G.F. KORSAKOVA. Reaktivnye izmeneniya v simmetrichnykh rogovitsakh u belykh krys (Reactive Changes in Both Corneas of White Rats). – Doklady Akademii Nauk SSSR, 81 (6): 1155. 1951.

338. ZHIRMUNSKAYA, E.A. O formirovanii i nervnoi regulyatsii tonusa zapiratel'nykh muskulov Anodonta cygnea (Formation and Nervous Regulation of Tonus of the Adductor Muscles of Anodonta cygnea). – Fiziologicheskii Zhurnal SSSR, 29 (5): 536. 1940.

339. ZHIRMUNSKII, A.V. Substantsional'nye i funktsional'nye izmeneniya pri denervatsii skeletnoi muskulatury mlekopitayushchikh (Substantial and Functional Changes on Denervation of Skeletal Muscles of Mammals). – Avtoreferat Dissertatsii, pp. 1-12. Leningrad. 1954.

340. ZHIRMUNSKII, A.V. Reperkussionnye izmeneniya v rogovitsakh posle pererezki sedalishchnogo nerva (Repercussional Changes in Both the Corneas after Cutting of the Sciatic Nerve). – Doklady Akademii Nauk SSSR, 100 (4): 793. 1955.

341. ZHIRMUNSKII, A.V. K voprosu o parabioticheskoi prirode reaktsii skeletnykh myshts mlekopitayushchikh na denervatsiyu (Parabiotic Nature of the Reaction of Skeletal Muscles of Mammals to Denervation). – Fiziologicheskii Zhurnal SSSR, 44 (6): 577. 1958.

342. ZHUKOV, E.K. Izmenenie labil'nosti gladkoi myshtsy kak faktor perekhoda klonicheskikh sokrashchenii v tonus (Change of Lability of Smooth Muscle as the Factor in Transformation of Clonic Contractions into Sustained Tonic Contractions). – Fiziologicheskii Zhurnal SSSR, 20 (1): 87. 1936a.

343. ZHUKOV, E.K. Izmenenie vyazko-elasticheskikh svoistv zapiratel'nykh myshts Anodonta i Unio pod vozdeistviem nervnoi sistemy (Change in Viscous Elastic Properties of Adductor Muscles of Anodonta and Unio under the Influence of the Nervous System). – Fiziologicheskii Zhurnal SSSR, 20 (1): 98. 1936b.

344. ZHUKOV, E.K. Nekotorye zakonomernosti evolyutsii vozbuzhdeniya (Certain Laws Governing Development of Excitation). – Zhurnal Obshchei Biologii, 7 (6): 435. 1946.

345. ZHUKOV, E.K. O prirode tonusa skeletnykh myshts (Nature of Tonus of Skeletal Muscles). – Nauchnyi Byulleten' Leningradskogo Universiteta, Vol. 24: 40. 1949.

346. ZHUKOV, E.K. Issledovaniya o tonuse skeletnykh myshts (Studies of the Tonus of Skeletal Muscles), pp. 1-241.—Leningrad. 1956.

347. ZHUKOV, E.K. and Z.N. DONTSOVA. Rol'obmena veshchestv v yavleniyakh usvoeniya ritma (Role of Metabolism in Phenomena of Acquisition of Rhythm). – Uchenye Zapiski Leningradskogo Universiteta, Vol. 6: 238. 1949.

348. ZHUKOV, E.K. and S.V. STREL'TSOVA. Toki deistviya v komissure anodonty (Action Currents in the Comissure of Anodonta). – Byulleten' Eksperimental'noi Biologii i Meditsiny, 15 (6): 61. 1943.

ALEXANDROW, W.Ya. Über die Bedeutung der oxydo-reduktiven Bedingungen für die vitale Färbung mit besonderen Berücksichtigung der Kernfärbung in lebendigen Zellen (Chironomus-Larven und Dafnia pulex). -Protoplasma, 17:161. 1932.

BABKIN, B.P., W.I. RUBASCHKIN, and W.W. SAWITSCH. Über die morphologischen Veränderungen der Pankreaszellen unter der Einwirkung verschiedenartiger Reize. - Arch. mikr. Anat. u. Entw. Gesch., Vol.74:68. 1909.

TSCHAGOWETZ, W.Y. Über die Rolle der semipermeablen Membranen bei Entstehung elektrischer Ströme im lebenden Gewebe. - Ztschr. Biol., Vol. 50:247. 1909.

CHAKHOTIN, S. Recherches physiologiques sur les protozoaires, faites au moyen de la micropuncture ultraviolette. - C. r. Acad. Sci. Paris, Vol. 200:2217. 1935.

TSCHIRIEW, S. Über die Nerven- und Muskelerregbarkeit. - Arch. Anat. u. Physiol. Abt. Vol. 1:489. 1877.

DORFMAN, W. Osmotic Behavior of the Sea-Urchin Egg as a Criterion of its Permeability. - Protoplasma, Vol. 19:578. 1933.

CHLOPIN, N. Experimentelle Untersuchungen über die sekretorischen Prozesse in Zytoplasma. I. Uber die Reaktion der Gewebselemente auf intravitale Neutralrotfärbung. - Arch. exper. Zellforsch. Vol. 4:462. 1927.

LEPESCHKIN, W. Kolloidchemie des Protoplasmas, pp. 2-228. - Berlin. 1924.

LEPESCHKIN, W. The Chemical and Physiological Composition of Protoplasm. - Science, Vol. 68:45. 1928.

LEPESCHKIN, W. My Opinion about Protoplasm. - Protoplasma, Vol. 9:269. 1930.

LEPESCHKIN, W. The "Vitaids". A Theory of the Fundamental Substances of Living Matter. - Biodynamics, Vol. 19:1. 1936.

LEPESCHKIN, W. Zell-Nekrobiose und Protoplasma-Tod. Protoplasma-Monographien. - Berlin, Vol. 12: 1-198. 1937.

LEPESCHKIN, W. Über Fluoid-Eigenschaften des Protoplasmas. - Protoplasma, Vol. 33:1. 1939.

MAKAROV, P. Experimentelle Untersuchungen an Protozoen mit Bezug auf das Narkose-Problem. - Protoplasma, Vol. 24:593. 1935.

MESTSCHERSKAJA, K.A. Experimentelle Untersuchungen an der Permeabilität der wachsenden Oocyten in den Insektenovarien. - Ztschr. Zellforsch. mikr. Anat., Vol. 13: 209. 1931.

NASSONOV, D. Das Golgische Binnennetz and seine Beziehungen zu der Sekretion (Fortsetzung). Morphologische und experimentelle Untersuchungen an einigen Säugetierdrüsen. - Arch. mikr. Anat. u. Entw. Mech., 100 (3/4):433. 1924.

NASSONOV, D. Die physiologische Bedeutung des Golgi-Apparats im Lichte der Vitalfarbungsmethode. - Ztschr. Zellforsch. mikr. Anat., Vol. 3:472. 1926.

NASSONOV, D. Über den Einfluss der Oxydations-prozesse auf die Verteilung von Vitalfarbstoffen in der Zelle. - Ztschr. Zellforsch. mikr. Anat. Vol. 11:179. 2930.

NASSONOV, D. Über die Ursachen der reversiblen Gelatinirung des Zellkerns. - Protoplasma, Vol. 15:239. 1932.

NASSONOV, D. Vitalfärbung des Makronukleus aerober und anaerober Infusorien. - Protoplasma. Vol. 17:218. 1932.

NASSONOV, D. Réaction du protoplasme vivant aux stimuli exterieurs (Paranecrose). XIII Congres international de zoologie, p. 202. - Paris. 1945.

NASSONOV, D.N. and W.A. ALEXANDROW. Uber die Ursachen der kolloidalen Veränderungen des Protoplasmas und der rehöhten Affinität des letzteren den Farbstoffen gegenüber unter dem Einfluss schädigender Agentien. - Acta zoologica, Vol. 24:189. 1934.

RESWJAKOFF, N.P. Zur Theorie der dekrementiellen Leitung im Nerven. - Pflug. Arch. Vol. 226:86. 1930.

JASSWOIN, G. Beiträge zur vergleichenden Histologie des Blutes und des Bindegewebes. IX. Experimentell-morphologische Studien über einige Zellformen des Lockeren Bindegewebes der Säugetiere. - Ztschr. mikr. - anat. Forsch., Vol. 29:513. 1930.

WERIGO, B. Die sekundaren Erregbarkeitsänderungen an der Kathode eines andauernd polarisierten Froschnerven. - Pflug. Arch. Vol. 31:417. 1883.

WERIGO, B. Zur Frage über die Beziehung zwischen Erregbarkeit und Leitungsfähigkeit des Nerven. - Pflug. Arch. Vol. 76:552. 1889.

WORONZOW, D. Über die Erregungsleitung im narkotisierten Nerv. - Pflug. Arch. Vol. 227:132. 1931.

Abelson P. H. and W. R. Duryee. 1949. Radioactive sodium permeability and exchange in frog eggs. Biol. Bull., 96 : 205.
Adrian E. D. 1933. The All- or Nothing Reaction. Ergebn. Physiol., 35 : 744.
Anson M. Z. and A. E. Mirsky. 1934. The equilibrium between active native trypsin and inactive denatured trypsin. Journ. Gen. Physiol., 17 : 393.
Apostolaki J. and R. Deriand. 1925. Changes in chronaxia with muscle degeneration. Proc. Soc. biol., 92 : 1482.
Arnett V. and W. S. Wilde. 1941. Potassium and water changes in excised nerve on stimulation. Journ. Neurophysiol., 4 : 572.
Arnold V. 1911. Eine Farbenreaktion von Eiweisskörpern mit Nitroprussidnatrium. Ztschr. physiol. Chem., 70 : 300.
Asmussen E. 1934. Untersuchungen über die mechanische Reaktion der Skelettmuskelfasern. Skand. Arch. Physiol., 70 : 233.
Auger D. 1933. Contribution à l'étude de la propagation de la variation électrique chez les Characées. C. r. Soc. biol., 113 : 1437.
Auger D. 1936. Comparaison entre la rythmicité des courants d'action cellulaires chez les végétaux et chez les animaux. Paris : 1—101.
Barnes T. C. and R. Beutner. 1941. The nature of the nerve impulse. Anat. Rec., 81, Suppl. : 42.
Basset J., M. A. Macheboeuf et G. Sandor. 1933. Étude sur les effets biologiques des ultrapressions. Action des pressions tres élevées sur les protéides. C. r. Acad. sci. Paris, 197 : 796.
Bear R. and F. Schmitt. 1939. Electrolytes in the axoplasm of the giant nerve fibers of the squid. Journ. Cell. Comp. Physiol., 14 : 205.
Bernstein J. 1906. Zur Frage der Präexistenztheorie oder Alterationstheorie des Muskelstroms. Pflüg. Arch., 113 : 605.
Bernstein J. 1912. Elektrobiologie. Braunschweig : 1—215.
Bernstein J. 1916. Ueber die Thermoströme des Muskels. Pflüg. Arch., 164 : 102.
Bernstein J. 1917. Ueber die elektrische Abteilung des Muskelquerschnittes. Pflüg. Arch., 166 : 201.
Bernstein J. und A. Tschermak. 1904. Ueber die Frage: Präexistenstheorie oder Alterationstheorie des Muskelstromes. Pflüg. Arch., 103 : 67.
Berwick M. C. 1951. The effect of anaesthetics on calcium release. Journ. Cell. Comp. Physiol., 38 : 95.
Beutner R. 1920. Entstehung elektrischer Ströme in lebenden Geweben und ihre künstliche Nachahmung durch syntetische organische Subetanzen. Stuttgart : 1—158.
Beutner R. 1933. Physical chemistry of living tissues and life processes as studied by artificial imitation of their single phases. Baltimore : 1—337.
Bialaszewicz K. 1929. Recherches sur répartition des électrolytes dans le protoplasme des cellules ovulaires. Protoplasma, 6 : 1.
Bialaszewicz K. 1933. Sur la détermination du volume de la phase dispersée dans les cellules vivants. Protoplasma, 19 : 350.
Blair H. A. 1935. Temperature coefficients in electrical excitation. Journ. Cell. Comp. Physiol., 6 : 291.
Blair E. A. and J. Erlanger. 1933. Comparison of characteristic of axons through their individual electrical responses. Amer. Journ. Physiol. 106 : 524.
Blair E. A. and J. Erlanger. 1936a. On excitation and depression in axons at the cathode of the constant current. Amer. Journ. Physiol., 114 : 317.
Blair E. A. and J. Erlanger. 1936b. Temporal summation in periferal nerve fibres. Amer. Journ. Physiol., 117 : 355.
Blumenthal A. 1896. Über die Wirkung verwandter chemischer Stoffe auf den quergestreiften Muskeln. Pflüg. Arch., 62 : 513.
Borsook H. 1950. Protein turnover and incorporation of labeled amino acids into tissue proteins in vivo and in vitro. Physiol. Rev., 30 : 206.
Borsook H., C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy. 1949 Uptake of labeled amino acids by tissue proteins in vitro. Federation Proc., 8 : 589.

---

* [The following bibliography has been photographically reproduced from the Russian original]

Boruttau H. und F. W. Fröhlich. 1904. Erregbarkeit und Leitfähigkeit der Nerven. Ztschr. allgem. Physiol., 4 : 153.
Bowditch H. P. 1871. Ueber die Eigenthümlichkeiten Reizbarkeit, welche die Muskelfasern des Herzens zeigen. Ber. über Verhandl. sächs. Ges. Wiss. Math.-Phys. Cl., 23 : 652.
Boyle P. J. and E. J. Conway. 1941. Potassium accumulation in muscle and associated changes. Journ. Physiol., 100 : 1.
Boyle P. J., E. J. Conway, F. Kane and H. L. O'Reilly. 1941. Volume of interfibre spaces in frog muscle and the calculation of concentrations in the fibre water. Journ. Physiol., 99 : 401.
Bridgman P. W. 1914. The coagulation of albumen by pressure. Journ. Biol. Chem., 19 : 511.
Bronk J. R., E. N. Harvey and F. H. Johnson. 1952. The effects of hydrostatic pressure on luminescent extracts of the ostracod crustacean, cypridina. Journ. Cell. Comp. Physiol., 40 : 347.
Brooks S. C. 1947. Permeability and enzyma reactions. Adv. Enzymology, 7 : 1.
Brooks S. C. and E. L. Chambers. 1954. The penetration of radioactive phosphate into morine eggs. Biol. Bull., 106 : 279.
Brown D. E. S. 1934. The effect of rapid changes in hydrostatic pressure upon the contraction of skeletal muscle. Journ. Cell. Comp. Physiol., 4 : 257.
Brown D. E. S. 1935. The liberation of energy in the contracture and simple twitch. Amer. Journ. Physiol., 113 : 20.
Brown D. E. S. 1936a. The effect of rapid compression upon events in the isometric contraction of skeletal muscle. Journ. Cell. Comp. Physiol., 8 : 141.
Brown D. E. S. 1936b. The sequence of events in the contraction of muscle at high pressure. Cold Sp. H. Symp. Q. B., 4 : 242.
Brown D. E. S. and D. J. Edwards. 1932. A contracture phenomenon in cross-striated muscle. Amer. Journ. Physiol., 101 : 15.
Brown D. E. S., F. H. Johnson and D. A. Marsland. 1941. The reversible denaturation of enzymes as a determining factor in the reaction of biological systems to temperature and pressure Anat. Ber., 81, Suppl. : 32.
Brücke E. Th. 1930. Vergleichende Physiologie des Erregungsvorganges. Ergebn. Biol., 6 : 327.
Brutkowski S. 1956. Krytyka fiziologicznej zasady «Wszystko albo-nic». Postery wiedzy medycznej, 1 : 3.
Bugnard L. and A. V. Hill. 1935. Electrical excitation of the fin nerve of sepia. Journ. Physiol., 83 : 425.
Bullock T., H. Grundfest, D. Nachmansohn, M. Rothenberg. 1947. Generality of role of acetylcholine in nerve and muscle conduction. Journ. Neurophysiol., 10 : 11.
Bullock T. H. and R. S. Turner. 1950. Events associated with conduction failure in nerve fibres. Journ. Cell. Comp. Physiol., 36 : 59.
Bungenberg de Jong H. G. 1932. Die Koazervation und ihre Bedeutung für die Biologie. Protoplasma., 15 : 110.
Bungenberg de Jong H. G. 1937a. Koazervation, I. Koll.-Ztschr., 79, 2 : 223; 3 : 334.
Bungenberg de Jong H. G. 1937b. Koazervation, II. Koll.-Ztschr., 80, 2 : 221; 3 : 350.
Bungenberg de Jong H. G. und H. Kruyt. 1930. Koazervation (Entmischung in kolloiden Systemen). Koll.-Ztschr., 50, 1 : 39.
Burdon-Sanderson J. and F. Gotch. 1891. Excitatory electrical change in muscle. Journ. Physiol., 12, Proc. Physiol. Soc., 5, XLIII—XLIV.
Cardot H. 1914. Variations des paramètres de l'excitabilité nerveuse en fonction de l'écartement des électrodes. C. r. Soc. biol., 77 : 276.
Cardot H. et H. Laugier. 1914. Influence de l'écartement des électrodes dans les mesures d'excitabilité. C. r. Soc. biol., 76 : 539.
Cattell McKeen. 1936. The physiological effects of pressure. Biol. Rev. Cambridge Philosoph. Soc., 11 : 441.
Cattell Mc K. and D. I. Edwards. 1928. The energy changes of skeletal muscle accompanying contraction under high pressure. Amer. Journ. Physiol., 86 : 371.
Cattell Mc K. and D. I. Edwards. 1932. Conditions modifying the influence of hydrostatic pressure on striated muscle, with special reference to the role of viscosity changes. Journ. Cell. Comp. Physiol., 1 : 11.
Chambers E. L. and W. E. White. 1954. The accumulation of phosphate by fertilized sea urchin eggs. Biol. Bull., 106 : 297.
Chambers L. A. and E. W. Flosdorf. 1936. The denaturation of proteins by sound nerves of audible frequencies. Journ. Biol. Chem., 114 : 75.

Chambers R. 1925. Études de microdissection. IV. Les structures mitochondriales et nucleaires dans les cellules germinales males chez la sauterelle. Cellule, 35 : 107.
Chambers R. and K. Höfler. 1931. Micrurgical studies of the tonoplast of Allium Cepa. Protoplasma, 12 : 338.
Chambers R. and G. Rényi. 1925. The structure of the cells in tissues as revealed by microdissection. Amer. Journ. Anat., 35 : 385.
Christiansen H. and E. Lynch. 1946. The conjugated non-protein, amino-acids of plasma. I. Postabsorptive concentrations of human plasma, serum and erythrocytes. Journ. Biol. Chem., 163 : 741.
Cohn W. and E. F. Cohn. 1939. Permeability of red corpuscles of the dog to sodium ion. Proc. Soc. Exper. Biol. Med., 41 : 445.
Cole K. S. 1933. Electric excitation in nerve. Cold Sp. H. Symp. Q. B., 1 : 131.
Colle J. 1933. Recherche de la relation qui lie la durée et l'intensité excitante liminaire d'un courant rectangulaire. Arch. intern. physiol., 37 : 410.
Colle J. 1934. Recherche de la forme de la courbe intensité—temps du ventricule de grenouille. C. r. Soc. biol., 115 : 207.
Conway E. and F. Kane. 1934. Diffusion equilibria for the frog's kidney. Biochem. Journ., 28 : 1760.
Cowan S. L. 1934. The action of potassium and other ions on the injury potential and action current in Maja nerve. Proc. Roy. Soc., Ser. B, 115 : 216.
Cremer M. 1906. Ueber die Ursache der elektromotorischen Eigenschaften der Gewebe, zugleich ein Beitrag zur Lehre von den polyphasischen Elektrolytketten. Ztschr. Biol., 47 : 561.
Cremer M. 1929. Erregungsgesetze des Nerven. Handb. norm. pathol. Physiol., 9 : 244.
Cuffler S. W. 1946. The relation of electric potential changes to contracture in skeletal muscle. Journ. Neurophysiol., 9 : 367.
Curtis H. J. and K. S. Cole. 1942. Membrane resting and action potentials from the squid giant axon. Journ. Cell. Comp. Physiol., 19 : 135.
Danielli J. 1936. Some properties of lipoid films in relation to the structure of the plasma membrane. Journ. Cell. Comp. Physiol., 7 : 393.
Danielli J. 1941. On the permeability change of stimulated nerve. Journ. Physiol., 100 : 117.
Davis H. 1923. The relationship of the «Chronaxie» of muscle to the size of the stimulating electrode. Journ. Physiol., 57 : 81.
Davis H. and A. Forbes. 1936. Chronaxie. Physiol. Rev., 16 : 407.
Davis H., A. Forbes, D. Brunswick and A. Hopkins. 1926. Studies of the nerve impulse. II. The question of decrement. Amer. Journ. Physiol., 76 : 448.
Davson H. 1934. Studies on the permeability of erythrocytes. Biochem. Journ., 28 : 676.
Dean R. B. 1941a. The determination of chloride in single isolated muscle fibres. Journ. Biol. Chem., 137 : 113.
Dean R. B. 1941b. Theories of electrolyte equilibrium in muscle. Biol. Symp., 3 : 331.
Dean R., T. Noonan, L. Haege and W. Fenn. 1941. Permeability of erythrocytes to radioactive potassium. Journ. Gen. Physiol., 24 : 352.
Dendrinos G. 1901. Ueber das Leitungsvermögen des motorischen Froschnerven in der Aethernarkose. Pflüg. Arch., 88 : 98.
Dervichian D. G. 1949. Coacervations as phase equilibrium in colloidal systems. Journ. Sci. a. its Applic., 2 : 210.
Deuticke H. I. 1930. Kolloidzustandsänderungen der Muskelproteine beim Absterben und bei der Ermüdung. Pflüg. Arch., 224 : 1.
Deuticke H. I. 1932. Kolloidzustandsänderungen der Muskelproteine bei der Muskeltätigkeit. Ztschr. physiol. Chem., 210 : 97.
Deuticke H. I. und U. Ebbecke. 1937. Über die chemischen Vorgänge bei der Kompressionsverkürzung des Muskels. Ztschr. physiol. Chem., 247 : 79.
Dow R. B. and J. Matthews. 1939. The disintegration of erythrocytes and denaturation of hemoglobin by high pressure. Philosoph. Mag., 27 : 637.
Draper M. H. and S. Weidmann. 1951. Cardiac resting and action potentials with an intracellular elektrode. Journ. Physiol., 115 : 74.
Du Bois-Reymond E. 1877. Gesammelte Abhandlungen zur allgemeinen Muskel- und Nervenphysik, Bd. 2. Leipzig : 1—753.
Dubuisson M. 1942. Sur la répartition des ions dans le muscle strite. Arch. intern. physiol., 52 : 439.
Duclaux J. 1934. Lecons de chimie physique appliquée à la biologie. III. Rigidité, thixotropie, concervation. Actualités scientif., Paris, 127 : 1.

D z i e m i a n A. J. 1939. The lipid content and the permeability of the erythrocyte. Journ. Cell. Comp. Physiol., 14 : 103.
D z i e m i a n A. J. 1942. The permeability and the lipid content of immature red cells. Journ. Cell. Comp. Physiol., 20 : 135.
E b b e c k e U. 1914. Wirkung allseitiger Kompression auf den Froschmuskel. Pflüg. Arch., 157 : 79.
E b b e c k e U. 1923. Der idiomuskuläre Wulst und seine Beziehung zu Dauerverkürzung und Errengungsleitung. Skand. Arch. Physiol., 43 : 138.
E b b e c k e U. 1927. Über das Gesetz der elektrischen Reizung und über die physikalische Bedeutung des Hoorwegschen Gesetzes und der Leitkonstante. Pflüg. Arch., 216 : 448.
E b b e c k e U. 1935a. Kompressionsverkürzung und idiomuskuläre Kontraktion und die Beziehung zwischen elektrischer und mechanischer Reizung. Pflüg. Arch., 236:662.
E b b e c k e U. 1935b. Muskelzuckung und Tetanus unter dem Einfluss der Kompression durch hohe Drucke. Pflüg. Arch., 236 : 669.
E b b e c k e U. 1936. Über das Verhalten der Querstreifung und des Muskelspertrums bei der Kompressionsverkürzung. Pflüg. Arch., 237 : 749.
E b b e c k e U. 1937. Über Kompression und Narkose. Pflüg. Arch., 238 : 441.
E b b e c k e U. 1944. Lebensvorgänge unter der Einwirkung hoher Drucke. Ergeb. Physiol., 45 : 34.
E b b e c k e U. und O. H a s e n b r i n g. 1935. Über die Kompressionsverkürzung des Muskels bei Einwirkung hoher Drucke. Pflüg. Arch., 236 : 405.
E b b e c k e U. und O. H a s e n b r i n g. 1936. Über die Spannleistung bei der Kompressinsverkürzung. Pflüg. Arch., 238 : 753.
E b b e c k e U. und H. S c h a e f e r. 1935. Über den Einfluss hoher Drucke auf den Aktionsstrom von Muskeln und Nerven. Pflüg. Arch., 236 : 678.
E d w a r d s D. J. and Mc. K. C a t t e l l. 1932. Measurements of the viscoelastic changes in muscle under pressure. Amer. Journ. Physiol., 101 : 31.
E f r o n J. 1885. Beiträge zur allgemeinen Nervenphysiologie. Pflüg. Arch., 36 : 467.
E g e R. 1920a. Über die Bestimmungen des Blutkörperchenvolumens. Biochem. Ztschr., 109 : 241.
E g e R. 1920b. Zur Frage der Permeabilität der Blutkörperchen gegenüber Glucose und Anelektrolyten. Arch. neerl. Physiol., 107 : 246.
E g e R 1920c. Die Verteilung der Glucose zwischen Plasma und roten Blutkörperchen. Zur Physiologie des Blutzuckers, IV. Biochem. Ztschr., 3 : 189.
E g e R. 1921a. Untersuchungen über das Volumen der Blutkörperchen in gegenseitig osmotischen Lösungen. Studien über das osmotische Verhältnis der Blutkörperchen, I. Biochem. Ztschr., 115 : 109.
E g e R. 1921b. Wie ist die Verteilung der Glucose zwischen den roten Blutkörperchen und der äussern Flüssigkeit zu erklären? Biochem. Ztschr., 114 : 88.
E g g l e t o n M. 1935. Diffusion of sugars into muscle. Journ. Physiol., 84 : 59.
E g g l e t o n Ph. 1930. The diffusion of creatine and urea through muscle. Journ. Physiol., 70 : 294.
E i c h l e r O. und K. S c h m e i s e r. 1951. Über die Aufnahme vor Ortho- und Pyrophosphat durch die Herzmuskelzelle, geprüft mit Radiophosphor P^{32}. Arch. exper. Pathol. u. Pharmakol., 212 : 255.
E i c h l e r W. 1929. Ueber die Erregbarkeitsmessund (absolute Schwellenspannung und den Einfluss des äußeren Widerstandes bei der Nervenreizung. Ztschr. Biol., 88 : 315.
E l l i o t t G., and G. H e v e s y. 1950. Turnover of phosphatides. Acta physiol. Scand., 19 : 370.
E m b d e n G. und E. A d l e r. 1922. Über die physiologische Bedeutung des Wechsels des Permeabilitätszustandes von Muskelfasergrenzschichten. Ztschr. physiol. Chem., 118 : 1.
E n g e l m a n n T. 1870. Beiträge zur allgemeinen Muskel- und Nervenphysiologie. I. Über die electrische Erregung der Ureter, mit Bemerkungen über die electrische Erregung im allgemeinen. Pflüg. Arch., 3 : 247.
E n g e l m a n n T. 1877. Ueber den Einfluss des Blutes und der Nerven auf das elektromotorische Verhalten künstlicher Muskelquerschnitte. Pflüg. Arch., 15 : 328
E p h r u s s i B. et A. N e u k o m m. 1927. L'ocuf d'Oursin, obéit-il à loi de Boyle — Mariotte? C. r. Soc. biol., 96 : 1291.
E r l a n g e r J., G. B i s h o p and H. G a s s e r. 1926. Experimental analysis of the simple action potential wave in nerve by the cathode ray oscillograph. Amer. Journ. Physiol., 78 : 537.
E r l a n g e r J. and E. A. B l a i r. 1931. The irritability changes in nerve in response to subthreshold constant currents and related phenomena. Amer. Journ. Physiol., 99 : 129.

Ernst E. 1928. Untersuchungen über Muskelkontraktion. VIII. Mitt. Wasserverschiebung als Grundlage der Zuckung. Mit einem Nachtrag über die osmotische Theorie der Kontraktion. Pflüg. Arch., 220 : 672.
Ernst E. und L. Csücs. 1929. Untersuchungen über Muskelkontraktion. IX. Mitt. Permeabilität und Tätigkeit. Pflüg. Arch., 223 : 663.
Ernst E. und J. Fricker. 1934a. Gebundenes oder freies Kalium im Muskel? Pflüg. Arch., 234 : 360.
Ernst E. und J. Fricker. 1934b. Permeabilität und Ionenkonzentration bei Muskelerregung. Pflüg. Arch., 234 : 399.
Ernst E. und L. Schaffer. 1928. Untersuchungen über Muskelkontraktion. VII. Mitt. Die Rolle des Kaliums in der Kontraktion. Pflüg. Arch., 220 : 655.
Ernst E. und I. Takács. 1931. Über die Rolle von Kalium. Phosphat und Milchsäure bei der Muskelkontraktion. Pflüg. Arch., 228 : 690.
Fabre and Fredet. 1925. Bull. Soc. Chem. Biol., 7 : 1071. Цит. по: D. Woodhouse and F. Pickworth, 1932.
Fahr G. 1909. Über den Natriumgehalt der Skelettmuskeln des Frosches. Ztschr Biol., 52 : 72.
Fenn W. O. 1931. The oxygen consumption of muscles made non-irritable by sugar solutions. Amer. Journ. Physiol., 97 : 635.
Fenn W. 1935. The diffusion of nitrogenous compounds from frog muscles in Ringer's solution. Journ. Cell. Comp. Physiol., 6 : 469.
Fenn W. 1936. Electrolytes in muscle. Physiol. Rev., 16 : 450.
Fenn W. 1937a. The rôle of tissue spaces in the osmotic equilibrium of frog muscle in hypotonic and hypertonic solutions. Journ. Cell. Comp. Physiol., 9 : 93.
Fenn W. 1937b. Loss of potassium in voluntary contraction. Amer. Journ. Physiol., 120 : 675.
Fenn W. O. 1938a. Factors affecting the loss of potassium from stimulated muscles. Amer. Journ. Physiol., 124 : 213.
Fenn W. 1938b. The potassium and water contents of cat nerves as affected by stimulation. Journ. Neurophysiol., 1 : 1.
Fenn W. O. 1939. The fate of potassium liberated from muscles during activity. Amer. Journ. Physiol., 127 : 356.
Fenn W. and D. Cobb. 1935a. The potassium equilibrium in muscle. Journ. Gen. Physiol., 17 : 629.
Fenn W. and D. Cobb. 1935b. Evidence for a potassium shift from plasma to muscles in response to an increased carbon dioxide tension. Amer. Journ. Physiol., 112 : 41.
Fenn W., D. Cobb., A. Hegnauer and B. Marsh. 1934. Electrolytes in nerve. Amer. Journ. Physiol., 110 : 74.
Fenn W. O., D. M. Cobb, J. F. Manery and W. R. Bloor. 1938. Electrolyte changes in cat muscle during stimulation. Amer. Journ. Physiol., 121 : 595.
Fenn W., D. Cobb. and B. Marsh. 1934. Sodium and chloride in frog muscle. Amer. Journ. Physiol., 110 : 261.
Fenn W. and L. Haege. 1942. The penetration of magnesium into frog muscle. Journ. Cell. Comp. Physiol., 19 : 37.
Fenn W., L. Haege, E. Sheridan and J. Flick. 1944. The penetration of ammonia into frog muscle. Journ. Gen. Physiol., 28 : 53.
Fenn W. O., T. R. Noonan, L. J. Mullins and L. Haege. 1941. The exchange of radioactive potassium with body potassium. Amer. Journ. Physiol., 135 : 149.
Feynn F. and M. Maizels. 1949. Cation control in human erythrocytes. Journ. Physiol., 110 : 301.
Fick A. 1863. Beiträge zur vergleichenden Physiologie der irritabelen Substanzen. Braunschweig : 1—68.
Fing E., E. E. Daniel and L. A. Woodbury. 1951. Effect of innervation and drugs upon direct membrane potentials of embryonic chick heart fibres. Journ. Pharm. Exp. Therap., 101 : 11.
Fischer A. 1935. Die Bindung von herapin an eiweiss. Biochem. Ztschr., 278 : 133.
Fischer M. and G. Moore. 1907. On the swelling of fibrin. Amer. Journ. Physiol., 20 : 330.
Fischer M. and W. Suer. 1935. Base-protein-acid compounds. Arch. Pathol., 20 : 683.
Fischer M. and W. Suer. 1938. Physiochemical state of protoplasm. Arch. Pathol., 26 : 51.
Fischer M. and W. Suer. 1939. Base-protein-scid compounds prepared from fibrin. Arch. Pathol., 27 : 811.

Flaig J. V. 1947. Viscosity changes in axoplasm under stimulation. Journ. Neurophysiol., 10 : 211.
Fleischmann W. 1928. Untersuchungen zur Frage der Permeabilität pflanzlicher und tierischer Zellmembranen für Kohlenhydrate. Pflüg. Arch., 220 : 448.
Folin O. and H. Berglund. 1922a. Some new observations and interpretations with reference to transportation, retention and excretion of carbohydrates. Journ. Biol. Chem., 51 : 213.
Folin O. and H. Berglund. 1922b. The retention and distribution of aminoacids with esperial reference to the urea formation. Journ. Biol. Chem., 51 : 375.
Folin O. and A. Svedberg. 1930. Diffusible non-protein constituents of blood and their distribution between plasma and corpuscles. Journ. Biol. Chem., 88 : 715.
Fontaine M. 1928a. De l'influence des fortes pressions sur les tissus musculaires immergés dans solutions hypertoniques. C. r. Soc. biol., 98 : 28.
Fontaine M. 1928b. Sur les anologies existant entre les effects d'une tetanisation et ceux d'une compression. C. r. Acad. Sci. Paris, 186 : 99.
Fontaine M. 1929. De l'influence de la durée et de l'intensité de la compression sur l'imbition de gastracnémien de grenouille. C. r. Soc. biol., 101 : 32.
Frédéricq H. 1928. Chronaxie; testing excitability bytmeans of time factor. Physiol. Rev., 8 : 501.
Fricke H. 1925. The electric capacity of suspensions with special reference to blood. Journ. Gen. Physiol., 9 : 137.
Fricke H., E. Parker and E. Ponder. 1939. Relative quantity of the fixed framework of the hemolyzed rabbit red cell. Journ. Cell. Comp. Physiol., 13 : 69.
Fröhlich F. W. 1904. Erregbarkeit und Leitfähigkeit des Nerven. Ztschr. allg. Physiol., 3 : 148.
Furchgott R. F. and E. Shorr. 1943. Phosphate exchange in resting cardiac muscle as indicated by radioactivity studies, IV. Journ. Biol. Chem. 151 : 65.
Furusawa K. 1929. The depolarization of crustacean nerve by stimulation or oxygen want. Journ. Physiol., 67 : 325.
Gad I. 1888. Ueber Trennung von Reizbarkeit und Leitungsfähigkeit des Nerven nach Versuchen des Hrn. Sawyer. Arch. Anat. Physiol. (Physiol. Abth.) : 395.
Gad I. 1889. Über Leitungsfähigkeit und Reizbarkeit der Nerven in ihren Beziehungen zur Längs- und Quererregbarkeit nach Versuchen des Hrn. Dr. Piotrowski. Arch. Anat. Physiol. (Physiol. Abth.) : 350.
Gallison W. E. 1931. The alleged presence of bound potassium in muscle. Journ. Biol. Chem., 90 : 665.
Garten S. 1901. Über rhythmische elektrische Vorgänge im quergestreiften Skelettmuskel. Abhandl. sächs. Ges. (Akad.) Wiss. Math.-Phys. Cl., 26 : 87.
Garten S. 1904. Experimentelle Nachprüfung der Untersuchung von Herrn Prof. Bernstein und Tschermak über die Frage der Präexistenztheorie oder Alterationstheorie des Muskelstromes. Pflüg. Arch., 105 : 291.
Gasser H. S. 1930. Contractures of skeletal muscle. Physiol. Rev., 10 : 35.
Gaunt W. E., H. D. Griffith and J. T. Irving. 1941. The accumulation of radio-active phosphorus following phosphorus deficiency in rats. Journ. Physiol., 100 : 372.
Gerard R. W. 1930. Response of nerve to oxygen lack. Amer. Journ. Physiol., 92 : 498.
Gerard R. W. 1932. Nerve metabolism. Physiol. Rev., 12 : 469.
Gerard R. W. and N. Tupikova. 1938. Creatine in nerve muscle and brain. Journ. Cell. Comp. Physiol., 12 : 325.
Gerard R. W. and N. Tupikova. 1939. Nerve and muscle phosphates. Journ. Cell. Comp. Physiol., 13 : 1.
Gold G. L. and A. K. Solomon. 1955. The transport of sodium into human erythrocytes in vivo. Journ. Gen. Physiol., 38 : 389.
Gotch F. and J. S. Macdonald. 1896. Temperature and excitability. Journ. Physiol., 20 : 247.
Graham J. and R. W. Gerard. 1946. Membrane potentials and excitation of single muscle fibers. Journ. Cell. Comp. Physiol., 28 : 99.
Granit R. and C. R. Scoglund. 1943. Accomodation and autorythmic mechanism in single sensory fibers. Journ. Neurophysiol., 6 : 337.
Greese R. 1954. Measurement of cation fluxes in rat diaphragm. Proc. Roy. Soc., Ser. B, 142 : 497.
Grijns G. 1896. Über den Einfluss gelöster Stoffe auf die roten Blutzellen in Verbindung mit den Erscheinungen der Osmose und Diffusion. Pflüg. Arch., 63 : 86.
Grundfest H. 1947. Bioelectric potentials in the nervous system and in muscle. Ann. Rev. Physiol., 9 : 477.

G u i l l i e r m o n d A. 1941. The cytoplasm of the plant cell. Publ. by Chron. Botan Co., Waltham. Mass : 1—248.
G u t s t e i n M. 1932. Zur Theorie der Vitalfärbung. Ztschr. Ges. exper. med., **82** : 479.
H a h n L. and G. H e v e s y. 1941a. Rate of penetration of ions through the capillary wall. Acta physiol. Scand., **1** : 347.
H a h n L. and G. H e v e s y. 1941b. Potassium exchange in the stimulated muscle. Acta physiol. Scand., **2** : 51.
H a h n L. and G. H e v e s y. 1942. Rate of penetration of ions into erythrocytes. Acta physiol. Scand., **3** : 193.
H a h n L., G. H e v e s y and O. R e b b e. 1939a. Permeability of corpuscles and muscle cells to potassium ions. Nature. **143** : 1021.
H a h n L. A., G. C. H e v e s y and O. H. R e b b e. 1939b. Do the potassium ions inside the muscle cells and blood corpuscles exchange with those present in the plasma? Biochem. Journ., **33** . 1549.
H a m b u r g e r H. J. 1889. Die Permeabilität der roten Blutkörperchen im Zusammenhang mit den isotonischen Coefficienten. Ztschr. Biol., **26** : 414.
H a m b u r g e r H. J. 1891. Über den Einfluss der Atmung auf die Permeabilität der Blutkörperchen. Ztschr. Biol., **28** : 405.
H a m i l t o n P. B. and D. D. V a n S l y k e. 1943. The gasometric determination of free amino acids in blood filtrates by the nonhydrin-carbon dioxide method. Journ. Biol. Chem., **150** : 231.
H a r r i s E. J. and M. M a i z e l s. 1951. The permeability of human erythrocytes to sodium. Journ. Physiol., **113** : 506.
H a r r i s E. J. and M. M a i z e l s. 1952. Distribution of ions in suspensions of human erythrocytes. Journ. Physiol., **118** : 40.
H a r r i s E. J. and T. A. J. P r a n k e r d. 1953. The rate of sodium extrusion from human erythrocytes. Journ. Physiol., **121** : 470.
H a s e n b r i n g O. 1940. Die Latentzeit bei der Kompressionsverkürzung (Druckzuckungen) des Muskels. Pflüg. Arch., **243** : 96.
H a u r o w i t z F. 1936. Ionenstruktur, Löslichkeit und Flockung der Proteine. (Ein Beitrag zur Systematik der Proteine). Kolloid-Ztschr., **74** : 208.
H a u r o w i t z F., F. D i - M o i a and S. T e k m a n. 1952. The reaction of native and denatured ovalbumin with congo red. Journ. Amer. Chem. Soc., **74** : 2265.
H ä u s l e r H. 1925a. Über Glukoseaufnahme durch Kinderblutkörperchen. Pflüg. Arch., **210** : 557.
H ä u s l e r H. 1925b. Über das Vorkommen zweier verschiedener Arten von Glukoseschwund bei Menschenblutkörperchen und ihre wechselseitigen Beziehungen. Pflüg. Arch., **210** : 561.
H e d i n S. G. 1897. Über die Permeabilität der Blutkörperchen. Pflüg. Arch., **68** : 229.
H e d i n S. G. 1898. Versuche über das Vermögen der Salze einiger Stickstoffbasen in die Blutkörperchen einzudringen. Pflüg. Arch., **70** : 525.
H e f f t e r A. 1907. Die reduzierenden Besthandtheile der Zelle. Jahrber. Thier. Chemie, **37** : 565.
H e i l b r u n n L. and P. H a m i l t o n. 1942. The presence of chloride in muscle fibers. Physiol. Zoology, **15** : 363.
H e i n b e c k e r P. 1929. Effect of anoxemia, carbon dioxide and lactic of electrical phenomena of myelinated fibers of the peripheral nervous system. Amer. Journ. Physiol., **89** : 58.
H e n d e r s o n P. 1907. Zur Thermodinamik der Flüssigkeitsnetten. Ztschr. physikal. Chem., **59** : 118.
H e p p e l L. A. 1939. The electrolytes of muscle and liver in potassium-depleted rats. Amer. Journ. Physiol., **127** : 385.
H e p p e l L. 1940. Effect of age and diet on electrolyte changes in rat muscle during stimulations. Amer. Journ. Physiol., **128** : 440.
H e r m a n n L. 1871. Weitere Untersuchungen über die Ursache der electromotorischen Erscheinungen an Muskeln und Nerven. Versuche über den Einfluss der Temperatur auf die electromotorischen Kraft des Muskelstromes. Pflüg. Arch., **4** : 163.
H e r m a n n L. 1874. Experimentelles und Kritisches über Electrotonus. Pflüg. Arch., **8** : 258.
H e r m a n n L. 1877. Untersuchungen über die Entwicklung des Muskelstroms. Pflüg. Arch., **15** : 191.
H e r m a n n L. 1886. Über die Ursache des Electrotonus. Pflüg. Arch., **38** : 153.
H e r m a n n L. 1905. Beiträge zur Physiologie und Physik des Nerven. Pflüg. Arch. **109** : 95.

Hertz H. 1947. Action potential and diameter of isolated nerve fibres under various conditions. Acta physiol. Scand., 13. Suppl. 43 : 1.
Hess A. and C. H. U. Chu. 1952. A histochemical study of fromman's striations and a qualitative determination of chloride in mammalian nerve fibers. Journ. Cell. Comp. Physiol., 39 : 31.
Hatényi G., B. Issekutz, Zs. Gáspár-Németh, I. Lichtenecker, M. Winter und M. Bedó. 1953. Kann Zucker von den Muskeln in die Blutbahn abgegeben werden? Acta physiol. Hung., Suppl. 4 : 52.
Hevesy G. C. 1951. The application of radioactive indicators in biochemistry. Journ. Chem. Soc.: 1618.
Hevesy G. and A. Aten. 1939. Interaction of plasma phosphate with the phosphorus compounds present in the corpuscles. Det. Kgl. Danske Videnskab. Selskab. Biol. Medd., 14 : 1.
Hevesy G. and L. Hahn. 1941. Exchange of cellular potassium. Det. Danske Videnskab. Selskab. Biol. Medd., 16 : 1.
Hevesy G. and J. Ottesen. 1943. Rate of formation of nucleic acid in the organs of the rat. Acta physiol. Scand., 5 : 237.
Hevesy G. and O. Rebbe. 1940. Rate of penetration of phosphate into muscle cells. Acta physiol. Scand., 1 : 171.
Hill A. 1930. The state of water in muscle and blood and the osmotic behaviour of muscle. Proc. Roy. Soc., Ser. B, 106 : 477.
Hill A. V. 1935. The intensity-duration relation for nerve excitation. Journ. Physiol., 83 : 30.
Hill A. V. 1936. Excitation and accomodation in nerve. Proc. Roy. Soc., Ser. B, 119 : 305.
Hill A. V., B. Katz and D. Y. Solandt. 1936. Nerve excitation by alternation current. Proc. Roy. Soc., Ser. B, 121 : 74.
Hill A. and P. Kupalov. 1930. The vapour presure of muscle. Proc. Roy. Soc., Ser. B, 106 : 445.
Hiruma K. 1923. Weitere Beobachtungen über Permeabilitätsänderungen in Lösungen von Nichtleitern. Pflüg. Arch., 200 : 497.
Höber R. 1905. Über den Einfluss der Salze auf den Ruhestrom des Froschmuskels. Pflüg. Arch., 106 : 599.
Höber R. 1907. Zur Kenntnis der Neutralsalzwirkungen. Beitr. chem. Physiol. Pathol., 11 : 35.
Höber R. 1909. Durchlässigkeit der Zellen für Farbenstoffe. Biochem. Ztschr., 20 : 56.
Höber R. 1926. Physikalische Chemie der Zellen und der Gewebe. 6-te Aufl. Leipzig : 1—955.
Höber R. 1936. Membrane permeability to solutes in its relations to cellular physiology. Physiol. Rev., 16 : 52.
Höber R. 1945. Physical chemistry of cells and tissues. Philadelphia: 1—676.
Hodgkin A. L. 1937. Evidence for electrical transmission in nerve. Journ. Physiol., 90 : 183.
Hodgkin A. L. 1938. The subthreshold potentials in a crustacean nerve fibre. Proc. Roy. Soc., Ser. B, 126 : 87.
Hodgkin A. L. 1939. The relation between conduction velocity and the electrical resistance outside a nerve fibre. Journ. Physiol., 94 : 560.
Hodgkin A. L. 1947. The membrane resistance of non-medullated nerve fibre. Journ. Physiol., 106 : 305.
Hodgkin A. L. 1951. The ionic basis of electrical activity in nerve and muscle. Biol. Rev., 26 : 339.
Hodgkin A. L. and A. F. Huxley. 1939. Action potentials recorded from inside a nerve fibre. Nature, 144 : 710.
Hodgkin A. L. and A. F. Huxley. 1945. Resting and action potential in single nerve fibres. Journ. Physiol., 104 : 176.
Hodgkin A. L. and A. F. Huxley. 1947. Potassium leakage from an active fibre. Journ. Physiol., 106 : 341.
Hodgkin A. L. and A. F. Huxley. 1953. Movement of radioactive potassium and membrane current in giant axon. Journ. Physiol., 121 : 403.
Hodgkin A. L. and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. Journ. Physiol., 108 : 37.
Hodgkin A. L. and R. D. Keynes. 1955. Active transport of cations in giant axons from Sepia and Loligo. Journ. Physiol., 128 : 28.
Hodgkin A. L. and W. A. H. Rushton. 1946. The electrical constants of a crustacean nerve fibre. Proc. Roy. Soc., Ser. B, 133 : 444.
Höfler K. 1918. Permeabilitätsbestimmung nach der plasmometrischen Methode. Ber. Dtsch. bot. Ges., 36 : 414.

Höfler K. 1930. Über Eintritts- und Rückgangsgeschwindigkeit der Plasmolyse und eine Methode zur Bestimmung der Wasserpermeabilität des Protoplasten. Jahrb. wiss. Bot., 73 : 300.
Höfler K. 1931. Das Permeabilitätsproblem und seine anatomische Grundlage Ber. Dtsch. bot. Ges., 49 : 79.
Höfler K. 1932. Zur Tonoplastenfrage. Protoplasma, 15 : 462.
Höfler K. 1934. Kappenplasmolyse und Salzpermeabilität. Ztschz. wiss. Mikrosk., 51 : 70.
Höfler K. 1939. Kappenplasmolyse und Ionenantagonismus. Protoplasma, 33 : 545.
Holleman L., H. G. Bungenberg de Jong und R. T. Modderman. 1934. Zur Kenntnis der lyophilen Kolloide. XXI. Mitt. Über Koazervation, 1: Einfache Koazervation von Gelatinesolen. Kolloid. Beih., 39 : 334.
Hoorweg I. L. 1892. Über die elektrische Nervenerregung. Pflüg Arch., 52 : 87.
Huber B. und K. Höfler. 1930. Die Wasserpermeabilität des Protoplasmas. Jahrb. wiss. Bot., 73 : 351.
Huxley A. F. and R. Stämpfli. 1949a. Evidence for saltatory conduction in peripheral myelinated nerve fibres. Journ. Physiol., 108 : 315.
Huxley A. F. and R. Stämpfli. 1949b. Saltatory transmission of the nervous impulse. Arch. Sci. Physiol., 3 : 435.
Huxley A. F. and R. Stämpfli. 1951. Direct determination of membrane resting potential and action potential in single myelinated nerve fibres. Journ. Physiol., 112 : 476.
Irving L. and J. F. Manery. 1936. The significance of the chlorides in tissues and animals. Biol. Rev., 11 : 287.
Jinnaka S. and R. Azuma. 1922. Electric currents as a stimulus with recept to its duration and strength. Proc. Roy. Soc., Ser. B, 94 49.
Johnson F. H., D. Brown and D. Marsland. 1942a. A basic mechanism in the biological effects of temperature, pressure and narcotics. Science, 95 : 200.
Johnson F. H., D. E. Brown and D. A. Marsland. 1942b. Pressure reversal of the action of certain narcotics. Journ. Cell. Comp. Physiol., 20 : 269.
Johnson F. H. and D. H. Campbell. 1945. The retardation of protein denaturation by hydrostatic pressure. Journ. Cell. Comp. Physiol., 26 : 43.
Johnson F. H. and D. H. Campbell. 1946. Pressure and protein denaturation. Journ. Biol. Chem., 163 : 689.
Johnson F. H. and H. Eyring. 1948. The fundamental action of pressure, temperature and drugs on enzymes, as revealed by bacterial luminescence. Ann. N. J. Acad. Sci., 49 : 376.
Johnson F. H., H. Eyring and R. W. Williams. 1942. The nature of enzyme inhibition in bacterial luminescence: sulfamide, urethane, temperature and pressure. Journ. Cell. Comp. Physiol., 20 : 247.
Johnson F. H. and G. G. Wright. 1946. Influence of hydrostatic pressure on the denaturation of staphylococcus antitoxin at 65° C. Proc. Nat. Acad. Sci., 32 : 21.
Juni E., M. Kamen, S. Spiegelman and J. Wiame. 1947. Physiological heterogeneity of metaphosphate in yeast. Nature, 160 : 717.
Kamen M. D. 1949. Tracer methods in biological research. Surv. Biol. Progress, 1 : 59.
Kamen M. D. and S. Spiegelman. 1948. Studies on the phosphate metabolism of some unicellular organisms. Cold. Sp. H. Symp. Q. B., 13 : 151.
Kato G. 1924. The theory of decrementless conduction in narcortised region of nerve. Tokyo : 1—166.
Kato G. 1934. The microphysiology of nerve. Tokyo : 1—139.
Katz B. 1936. Neuro-muscular transmission in crabs. Journ. Physiol., 87 : 199.
Katz B. 1937. Experimental evidence for non-conducted response of nerve to subthreshold stimulation. Proc. Roy. Soc., Ser. B, 124 : 244.
Katz B. 1939. Electric excitation of nerve. London : 1—150.
Katz B. and O. H. Schmitt. 1940. Electric interaction between 2 adjacent nerve fibres. Journ. Physiol., 97 : 471.
Keil F. 1922. Über das Gesetz der Streckenlänge bei der Nervenreizung. Ztschr. Biol., 75 : 1.
Keth N. M. and M. H. Power. 1937. The urinary excretion of sucrose and its distribution in the blood after intravenous injection into normal men. Amer. Journ Physiol., 120 : 203.
Keynes R. D. 1951a. The leakade of radioactive potassium from stimulated nerve. Journ. Physiol., 113 : 99.
Keynes R. D. 1951b The ionic movements during nervous activity. Journ. Physiol 114 119.

Keynes R. D. 1954. The ionic fluxes in frog muscle. Proc. Roy. Soc., Ser. B, 142 :369.
Keynes R. D. and P. R. Lewis. 1951a. The sodium and potassium content of cephalopod nerve-fibres. Journ. Physiol., 114 : 151.
Keynes R. D. and P. R. Lewis. 1951b. The resting exchange of radioactive potassium in crab nerve. Journ. Physiol., 113 : 73.
Keynes R. D. and G. W. Mruisel. 1954. The energy requirement for sodium extrusion from a frog muscle. Proc. Roy. Soc., Ser. B, 142 : 383.
Klotz I. M. and J. M. Urquhart. 1949. The binding of organic ions by proteins. Comparison of native and of modified proteins. Journ. Amer. Chem. Soc., 71 : 1597.
Knake Ch. 1927. Bindegewebsstudien. III. Die Histo- und Leucocyten-Entstehung bei Tuschen-Wirkung auf das lockere Bindegewebe des Kaninchens. Ztschr. Zellforsch. mikr. Anat., 5 : 208.
Koeppe H. 1897. Der osmotische Druck als Ursache des Stoffaustausches zwischen roten Blutkörperchen und Salzlösungen. Pflüg. Arch., 67 : 189.
Korr I. M. 1939. Oxidation-reductions in heterogeneous systems. Cold. Sp. H. Symp. Q. B., 7 : 74.
Kozawa Sh. 1913. Eine Sonderstellung der menschlichen Blutkörperchen in der Durchlässigkeit für Monosaccharide. Zbl. Physiol., 27 : 2.
Kozawa Sh. 1914. Beiträge zum arteigenen Verhalten der roten Blutkörperchen. III. Artdifferenzen in der Durchlässigkeit der roten Blutkörperchen. Biochem. Ztschr., 60 : 231.
Kramer B. and F. Tisdall. 1922. The distribution of sodium, potassium, calcium and magnesium between the corpuscles and serum of human blood. Journ. Biol. Chem., 53 : 241.
Krigsman B. J., J. C. van der Kamer, E. W. van Lennep und A. Stolk. 1950. Untersuchungen über die Aktivität des peripherenn Nervensystems bei verschiedener Temperatur. Acta physiol.-pharmacol. Neerl., 1 : 63.
Krogh A. 1943. The exchange of ions between cells and extracellular fluid. I. The uptake of potassium into the chorion membrane from the hens egg. Acta physiol. Scand., 6 : 203.
Krogh A. 1946. The active and passive exchanges of inorganic ions through the surface of living cells and through living membranes generally. Proc. Roy. Soc., Ser. B, 133 : 141.
Krogh A. and A. L. Lindberg. 1944. The exchange of ions between cells and extracellular fluid. III. The exchange of sodium with glucose in the frog's heart. Acta physiol. Scand., 7 : 238.
Krogh A., A. L. Lindberg and B. Schmidt-Nielsen. 1944. The exchange of ions between cells and extracellular fluid. II. The exchange of potassium and calcium between the frog heart muscle and the bathing fluid. Acta physiol. Scand., 7 : 221.
Kronfeld P. und F. Scheminsky. 1926. Beiträge zur physikalisch-chemischen Biologie der Forellentwicklung. 2-te Mitt. Wachstum, Dotter Resorption und Wasserhaushalt. Arch. Entw. Mech., 107 : 129.
Krouse R. and W. E. Burge. 1936. A study of the cause of electrical phenomena exhibited in animals and plants. Amer. Journ. Physiol., 116, Proc. 94.
Kühne W. 1864. Untersuchungen über das Protoplasma und die contractilität. Leipzig : 1—158.
Kunitz M. 1948. The kinetics and thermodynamics of reversible denaturation of crystalline soybean tripsin inhibitor. Journ. Gen. Physiol., 32 : 241.
Lambert E. T., B. F. Skinner, A. Forbes. 1933. Changes in rheobase and chronaxie on sectioning motor nerve. Amer. Journ. Physiol., 105 : 65.
Lanczos A. 1930. Über die Gültigkeit des «Alles oder Nichts» Gesetzes der Erregung. Pflüg. Arch., 223 : 709.
Lapicque L. 1909. Definition experimentale de l'excitabilité. C. r. Soc. biol. Paris, 67 : 280.
Lapicque L. 1926. L'excitabilité en fonction du temps. Paris : 1—365.
Lapicque L. m-r et m-me. 1907. Influence d'une variation locale de température sur l'excitabilité du nerf moteur. C. r. Soc. biol., 59 : 37.
Lapicque M. 1923. Action des centres encéphaliques sur la chronaxie des nerfs moteurs. C. r Soc. biol., 88 : 46.
Laufler M. A. and R. B. Dow. 1941. The denaturation of tabacco mosaic virus high pressure. Journ. Biol. Chem., 140 : 509.
Lillie R. S. 1936. The passive iron wire model of protoplasmic and nervous transmission and its physiological analogies. Biol. Rev., 11 : 181.
Lindberg O. 1950. On surface reactions in the sea urchin egg. Exper. Cell. Research. 1 : 105.

Lodholz E. 1913. Über die Gültigkeit des «Alles oder Nichts Gesetzes» für die markhaltige Nervenfaser. Ztschr. allg. Physiol., 15 : 269.
Loeb J. 1901. On an apparently new form of muscular irritability (contact irritability) produced by solutions of salts (preferably sodium salts) whose anions are liable to form insoluble calcium compounds. Amer. Journ. Physiol., 5 : 362.
Lorente de No R. 1947. A study of nerve physiology. Studies from the Rockefeller Institute, 131 : 3; 132 : 1.
Lucas K. 1905. On the gradation of activity in a skeletal muscle fibre. Journ. Physiol., 33 : 125.
Lucas K. 1907. The analysis of complex excitable tissues by their response to electric currents of short duration. Journ. Physiol., 35 : 310.
Lucas K. 1909. The «all or none» contraction of the amphibian skeletal muscle fibre. Journ. Physiol., 38 : 113.
Lucas K. 1911. On the transference of the propagated disturbance from nerve to muscle with special reference to apparent inhibition described by Wedensky. Journ. Physiol., 43 : 46.
Lucas K. 1913. The effect of alcohol on the excitation, conduction and recovery processes in nerve. Journ. Physiol., 46 : 470.
Lucas K. 1917. The conduction of nervous impulse. London: 1—113.
Lucas K. and G. Mines. 1907—1908. Temperature and excitability. Journ. Physiol., 36 : 334.
Lucké B. 1931. The effect of certain narcotics (uretanes) on permeability of living cells to water. Biol. Bull., 60 : 72.
Lucké B. 1940. The living cell as an osmotic system and its permeability to water. Cold. Sp. H. Symp. Q. B., 8 : 123.
Lucké B., H. Hartline and R. Ricca. 1939. Comparative permeability to water and to certain solutes of the egg cells of three marine invertebrates (Arbacia, Gumingia and Chaetopterus). Journ. Cell. Comp. Physiol., 14 : 237.
Lucké B., M. Larrabee and K. Hartline. 1935/36. Studies on osmotic equilibrium and on the kinetic of osmosis in living cells by a diffraction method. Journ. Gen. Physiol., 19 : 1.
Lucké B. and M. Mc Cutcheon. 1927. The effect of salt concentration of the medium on the rate of osmosis of water through the membrane of living cells. Journ. Gen. Physiol., 10 : 665.
Lucké B., R. Ricca and K. Hartline. 1936. Comparative permeability to water and certain solutes of the egg cells of three marine unvertebrates (Arbacia, Cumingia and Chaetopterus). Biol. Bull., 71 : 397.
Lund E. J. 1928. Relation between continuous bioelectric currents and cell respiration, II a. III. Journ. Exper. Zool., 51 : 265.
Lundegardh H. 1940. Investigation as the absorption and accumulation of inorganic ions. Lantbr. Högsk. Ann., 8 : 233.
Lundsgaard E. 1930a. Untersuchungen über Muskelkontraktion ohne Milchsäurebildung. Biochem. Ztschr., 217 : 162.
Lundsgaard E. 1930b. Die Monojodessigsäurewirkung auf die enzymatische Kohlenhydratspaltung. Biochem. Ztschr., 220 : 1.
Lundsgaard E. 1930c. Weitere Untersuchungen über Muskelkontraktionen ohne Milchsäurebildung. Biochem. Ztschr., 227 : 51.
Lundsgaard E. 1934. Phosphogen und Pyrophosphatumsatz in jodessigsäurevergifteten Muskeln. Biochem. Ztschr., 269 : 308.
Lundsgaard C. and S. Holböll. 1926. Untersuchungen über die Verteilung einiger Nichtelektrolyte auf Blutkörperchen und Plasma des Menschenblutes. Biochem. Ztschr., 173 : 269.
Maizels M. 1943. Phosphate base and haemolysis in stored blood. Quart. Journ. Exper. Physiol., 32 : 143.
Maizels M. 1945. Intercellular plasma in the centrifuged erythrocytes of normal human blood. Quart. Journ. Exper. Physiol., 33 : 129.
Maizels M. 1949. Catiol control in human erythrocytes. Journ. Physiol., 108 : 247.
Maizels M. 1951. Factors in the active transport of cations. Journ. Physiol., 112 : 59.
Maizels M. 1954. Cation transport in chicken erythrocytes. Journ. Physiol., 125 : 263.
Manery J. F. and W. Bale. 1941. The penetration of radioactive sodium and phosphorus, into the extra- and intracellular phases of tissues. Amer. Journ. Physiol., 132 : 215.
Mangold E. 1923. Reiz und Erregung, Reizleitung und Erregungsleitung. Ergebn. Physiol., 21 : 361.
Marcuse A. J. 1877. Über die Abhängigkeit der Erregung von der Länge der elektrish durchstromten Nervenstrecke. Phys.-Med Verhandl., 10 : 158.

Masing E. 1913. Sind die roten Blutkörper durchgängig für Traubenzucker? Pflüg. Arch., 149 : 227.
Masing E. 1914a. Über die Durchgängigkeit menschlicher Blutkörper für Zucker. Pflüg. Arch., 159 : 476.
Masing E. 1914b. Über die Verteilung von Traubenzucker im Menschenblut und ihre Abhängigkeit von der Temperatur. Pflüg. Arch., 156 : 401.
Mayer A. 1911. Versuche zur Frage der thermischen Erregung. Ztschr. Biol., 57 : 507.
McCutcheon M. and B. Lucké. 1927. The kinetics of exosmosis of water from living cells. Journ. Gen. Physiol., 10 : 659.
McCutcheon M. and B. Lucké. 1932. The living cell as an osmotic system and its permeability to water. Physiol. Rev., 12 : 68.
Meyer K. et I. Sievers. 1936. La permeabilité des membranes, I, II, III, IV. Helvet. Chen. Acta, 19 : 649, 665, 948, 987.
Michaelis L. 1925. Contribution to the theory of permeability of membranes for electrolytes. Journ. Gen. Physiol , 8 : 33.
Michaelis L. 1926. Die Permeabilität von Membranen. Naturwiss., 14 : 33.
Michaelis L., A. Fujita und Sh. Dokan. 1925. Untersuchungen über elektrische Erscheiningen und Ionendurchlässigkeit von Membranen. Biochem. Ztschr., 158 : 11; 159 : 370; 161 : 47; 162 : 245; 164 : 23.
Michaelis L., R. Ellsworth and A. Weech. 1927. Studies on the permeability of membranes. II. Determination of ionic transfer numbers in membranes from concentration chains. Journ. Gen. Physiol., 10 : 671.
Michaelis L. and W. Perlzweig. 1927. Studies on permeability of membranes. I. Introduction and the diffusion of ions across the dried collodion membrane. Journ. Gen. Physiol , 10 : 575.
Michaelis L., A. Weech and A. Yamatori. 1927. Studies on the permeability of membranes. III. Electric transfer experiments with the dried collodion membrane. Journ. Gen. Physiol., 10 : 685.
Mirsky A. E. 1938. Protein denaturation. Gold Sp. H. Symp. Q. B., 6 : 150.
Möllendorff M. 1927. Bindegewebesstudien. VI. Die Wirkung der künstlichen Höhensonnenabstrahlung auf das subkutane Bindegewebe der weissen Maus. Ztschr. Zellforsch. mikr. Anat., 6 : 151.
Möllendorff W. 1927. Bindegewebesstudien. V. Die Ableitung der entzündlichen Gewebsbilder aus einer den Bindegeweben gemeinsamen Zellbildungsfolge. Ztschr. Zellforsch. mikr. Anat., 6 : 61.
Möllendorff W. und M. Möllendorff. 1926. Die Fibrocytennetz im lockeren Bindegewebe seine Wandlungsfähigkeit und Antheilnahme am Stoffwechsel. Ztschr. Zellforsch. mikr. Anat., 3 : 503.
Mond R. 1930. Weitere vergleichende Untersuchungen über Membranstruktur und Permeabilität der roten Blutkörperchen. Pflüg. Arch., 224 : 161.
Mond R. und K. Amson. 1928. Über die Ionenpermiabilität des quergestreiften Muskels. Pflüg. Arch., 220 : 67.
Mond R. und F. Hoffmann. 1928. Weitere Untersuchungen über die Membranstruktur der roten Blutkörperchen. Die Beziehungen zwischen Durchlässigkeit und Molekularvolum. Pflug. Arch., 219 : 467.
Mond R. und H. Netter. 1930. Ändert sich die Ionenpermeabilität des Muskels während seiner Tätigkeit? Pflüg. Arch., 224 : 702.
Mond R. und H. Netter. 1932. Über die Regulation des Natriums durch den Muskel. Pflüg. Arch., 230 : 42.
Monnier A. M. 1934. L'excitation électrique des tissus: Essai d'interprétation physique. Paris : 1—326.
Monnier A. M. et H. H. Jasper. 1932. Relation entre la vitesse de propagation de l'influx nerveux et la chronaxie de subordination. C. r. Soc. biol., 110 : 286.
Moore B. and H. E. Roaf. 1908. On the equilibrium between the cell and its environment in regard to soluble constituents. Biochem. Journ., 3 : 55.
Moore B. und H. Roaf. 1913. Der osmotische Druck der Kolloide und seine biologische Bedeutung. Koll.-Ztschr., 13 : 133.
Moore B., H. Roaf and I. Webster. 1912. Biochem. Journ., 6 : 110. (Цит. по: L. Heilbrunn, 1952).
Mueller C. B. and A. B. Hastings. 1951. The rate of transfer of phosphorus across the red blood cell membrane. Journ. Biol. Chem., 189 : 869.
Mullins L. J. 1942a. Selective accumulation of potassium by myosin. Federation Proc., 1 : 61.
Mullins L. J. 1942b. Permeability of yeast cells to radiophosphate. Biol., 83 : 326.
Mullins L. J., W. O. Fenn, T. R. Noonan and L. Haege. 1941. Permeability of erythrocytes to radio active potassium. Amer. Journ. Physiol., 135 : 93.

Nachmansohn D. 1940. On physiological significance of choline esterase. Yale Journ. Biol. Med., 12 : 565.
Nachmansohn D. 1946. Chemical mechanism of nervous action currents in biochemical research. New York: 1—335.
Nastuk W. L. and A. L. Hodgkin. 1950. The electrical activity of single muscle fibres. Journ. Cell. Comp. Physiol., 35 : 39.
Nathansohn A. 1904a. Über die Regulation der Aufnahme anorganischer Salze durch die Knollen von Dahlia. Jahrb. wiss. Bot., 39 : 607.
Nathansohn A. 1904b. Weitere Mitteilungen über die Regulation der Stoffaufnahme Jahrb. wiss. Bot., 40 : 403.
Nernst W. 1892. Über die Löslichkeit von Mischkrystallen. Ztschr. physikal. Chem., 9 : 137.
Nernst W. 1899. Zur Theorie der elektrischen Reizung. Nachr. Ges. Wiss. Göttingen, Math.-Phys. Cl., 1 : 104.
Nernst W. 1908. Zur Theorie des elektrischen Reizes. Pflüg. Arch., 122 : 275.
Netter H. 1927. Über die Ruheströme des Nerven und die Ionenpermeabilität einer Hülle. Pflüg. Arch., 218 : 310.
Netter H. 1928. Über die Elektrolytgleichgewichte an elektiv ionenpermeabelen Membranen und ihre biologische Bedeutung. Pflüg. Arch., 220 : 105.
Netter H. 1934. Die Stellung des Kaliums im Elektrolytsystem des Muskels. Pflüg. Arch., 234 : 680.
Neuschlosz S. M. 1923. Über die Bedeutung der K-Ionen für den Tonus des quergestreiften Skelettmuskels. I. Mitt. Pflüg. Arch., 199 : 410.
Neuschlosz S. M. 1925a. Über die Bedeutung der K-Ionen für den Tonus des quergestreiften Skelettmuskels. III. Mitt. Die Beziehungen des Erregungscontractur zum Gehalt des Muskels an gebundenen Kalium. Pflüg. Arch., 207 : 27.
Neuschlesz S. M. 1925b. Über die Bedeutung der K-Ionen für den Tonus des quergestreiften Skelettmuskels. IV. Mitt. Die chemischen Vorgänge bei der Kalicontractur des isolierten Kaltblütermuskels. Pflüg. Arch., 207 : 37.
Neuschlosz S. M. 1926a. Untersuchungen über die Kaliumbindung in der Kammermuskulatur und ihre Bedeutung für die Herzfunktion. Pflüg. Arch., 213 : 19.
Neuschlosz S. M. 1926b. Über die Bedeutung der K-Ionen für den Tonus des quergestreiften Skelettmuskels. V. Mitt. Die tonische Komponente des Strychnintetanus und ihre Beeinflussung durch peripher angreifende Agenzien. Pflüg. Arch., 213 : 40.
Neuschlosz S. M. 1926c. Über die Bedeutung der K-Ionen für den Tonus des quergestreiften Skelettmuskels. VI. Mitt. Über den Einfluss der Elektrolyte der Spülflüssigkeit auf den Gehalt der Muskeln an gebundenen Kalium. Pflüg. Arch., 213 : 47.
Neuschlosz S. M. und R. A. Trelles. 1924. Über die Bedeutung der K-Ionen für den Tonus des quergestreiften Skelettmuskels. II. Mitt. Über die Menge und die Bindungsweise des Kaliums in quergestreiften Muskeln unter normalen und pathologischen Bedingungen. Pflüg. Arch., 204 : 374.
Neuschlosz S. M. und K. Walter. 1926. Über die Bedeutung der K-Ionen für den Tonus des quergestreiften Skelettmuskels. VII. Mitt. Über die physikochemischen Bedingungen der Ionenbindung an hydrophile Gele. Pflüg. Arch., 213 : 58.
Noonan T., W. Fenn and L. Haege. 1941a. The distribution of injected radioactive potassium in rats. Amer. Journ. Physiol., 132 : 474.
Noonan T., W. Fenn and L. Haege. 1941b. The effects of denervation and of stimulation on exchange of radioactive potassium in muscle. Amer. Journ. Physiol., 132 : 613.
Oker-Blom M. 1901. Tierische Säfte und Gewebe in physikalisch-chemischer Beziehung. IV. Mitt. Die elektromotorischen Erscheinungen an ruhenden Froschmuskeln. Pflüg. Arch., 84 : 191.
Orskov S. L. 1946. Solvent water in the human erythrocytes. Acra physiol. Scand., 12 : 192.
Osterhout W. J. V. and S. E. Hill. 1930. Salt bridges and negative variations. Journ. Gen. Physiol., 13 . 547.
Ostwald W. 1890. Elektrische Eigenschaften halbdurchlässiger Scheidewände. Ztschr. physikal. Chem., 6 : 71.
Overton E. 1895. Über die osmotischen Eigenschaften der lebenden Pflanzen und Tierzelle. Vierteljahrschr. Naturforsch. Ges. Zürich., 40 : 159.
Overton E. 1896. Über die osmotischen Eigenschaften der Zelle in ihrer Bedeutung für die Toxikologie und Pharmakologie. Vierteljahrschr. Naturforsch. Ges. Zürich., 41 : 388.

Overton E. 1902. Beiträge zur allgemeinen Muskel- und Nervenphysiologie. I. Mitt. Pflüg. Arch., 92 : 115.
Page J. H. 1927. The electrolytic content of the sea urchin and star fisch egg. Biol. Bull., 52 : 168.
Paine S. 1911. The permeability of yeast-cell. Proc. Roy. Soc., Ser, B, 84 : 289.
Parpart A. and J. Shull. 1935a. Solvent water in the normal mammalian erythrocyte. Journ. Cell. Comp. Physiol., 6 : 137.
Parpart A. and J. Shull. 1935b. Permeability of the erythrocyte for glycerol. Journ. Cell. Comp. Physiol., 6 : 129.
Pauli W. und I. Matula. 1916. Der Thermostrom des Muskels. Pflüg. Arch., 163 : 355.
Pfeffer W. 1877. Osmotische Untersuchungen. Leipzig : 1—236.
Pflüger E. 1859. Physiologie des Electrotonus. Berlin : 1—502.
Pond S. E. 1921. Correlation of propagation velocity of contraction-wave in muscle with electrical conductivity of surrounding medium. Journ. Gen. Physiol., 3 : 807.
Ponder E. 1934. The mammalian red cell and the properties of haemolytic systems. Protoplasma-Monograph., 6. Berlin: 1—311.
Porter R. R. 1948. The unreactive amino groups of proteins. Bioch.-biophys. acta, 2 : 105.
Prosser C. L. and A. H. Chambers. 1938. Excitation of nerve fibers in the squid (Loligo pealii). Journ. Gen. Physiol., 21 : 781.
Pumphrey R. J., O. H. Schmitt and J. Z. Young. 1940. Correlation of local excitability with local physiological responses in the giant axon of the squid (Loligo). Journ. Physiol., 98 : 47.
Putnam F. W. 1953. Protein Denaturation. In: The Proteins. Chemistry biological activity and methods, 1. New York : 1—807.
Quincke G. 1898. Über Oberflächenspannung des reinen Goldes. Ann. Physik, 64 : 618.
Reginster A. 1937. Recherches sur la potassium diffusible e non diffusible du muscle. Arch. intern. Physiol., 45 : 69.
Reginster A. 1938a. Recherches sur la rôle du potassium dans les phénomènes d'excitation. Arch. intern. Physiol., 47 : 24.
Reginster A. 1938b. Nouvelles recherches sur la dissociation du complexe organo-potassique du muscle au cours des phénomènes d'excitation. Arch. intern. Physiol., 67 : 71.
Renquist J., V. Leskinen und S. Parviainen. 1931. Die Bedeutung des Parameters *a* der Weiss-Hoorwegschen Reizformel. Skand. Arch. Physiol., 61 : 113.
Ritchie A. D. 1932. The «all-or-none» principle. Biol. Rev. Cambr. Phylos. Soc., 7 : 336.
Roaf H. E. and E. Alderson. 1907. On the effect of narcotic agents in the detachment of electrolytes from cell proteins. Biochem. Journ., 2 : 412.
Roncato A. 1923. Azione dell'urea sui globuli rossi nucleati. Arch. di scienze biol., 5 : 44.
Ronzoni E. 1931. The source of energy of nerve activity. Journ. Biol. Chem., 92 : 111.
Rosenberg H. 1935. Excitation of medullated and non-medullated nerves by currents of short duration. Journ. Physiol., 84 : 50.
Rosenberg Th. and W. Wilbrandt. 1952. Enzymatic processes in cell membrane penetration. Intern. Rev. Cytol., 1 : 65.
Rosenblueth A. 1952. The local responses of axons. Ergebn. Physiol. Exper. Pharmacol., 47 : 24.
Rosenblueth A. and J. Garcia Ramos. 1951. The local responses of mammalian spinal roots to alternating sinusoidal currents. Journ. Cell. Comp. Physiol., 38 : 321.
Rosenblueth A. and J. Garcia Ramos. 1952. A further study of the local responses of axons. Journ. Cell. Comp. Physiol., 39 : 109.
Rosenblueth A. and J. V. Luco. 1950. The local responses of myelinated mammalian axons. Journ. Cell. Physiol., 36 : 289.
Rotschuh K. E. 1955. Analyse der Bedingungen für die fortschreitenden Desorganisationskontraktur verletzer Skelettmuskelfasern. Pflüg. Arch., 261 : 557.
Ruhland W. 1908. Die Bedeutung der Kolloidnatur wässeriger Farbstofflösungen für ihr Eindringen in lebende Zellen. Ber. Dtsch. bot. Ges., 26a : 772.
Ruhland W. 1909a. Beiträge zur Kenntnis der Permeabilität der Plasmahaut. Jahrb. wiss. Bot., 46 : 1.
Ruhland W. 1909b. Zur Frage der Ionenpermeabilität. Ztschr. Bot., 1 : 747.

26*

Ruhland W. 1911. Untersuchungen über den Kohlenhydratstoffwechsel in Beta vulgaris. Jahrb. wiss. Bot., 50 : 200.
Ruhland W. 1912a. Plasmahaut als Ultrafilter bei det Kolloidaufnahme. Ber. Dtsch. bot. Ges., 30 : 139.
Ruhland W. 1912b. Studien über die Aufnahme von Kolloiden durch die pflanzliche Plasmahaut. Jahrb. wiss. Bot., 51 : 376.
Ruhland W. 1913. Kolloidchemische Protoplasmastudien. Koll. Ztschr., 12 : 113.
Ruhland W. und C. Hoffman. 1925. Die Permeabilität von Beggiatoa mirabilis. Ein Beitrag zur Ultrafiltertheorie des Plasmas. Planta, 1 : 1.
Ruiter L. and H. G. Bungenberg de Jong. 1947. Interfacial tension of gum arabic-gelatin complex coacervates and their equilibrium liquids. Proc. Roy Akad. Amsterdam, 50 : 836.
Rushton W. A. H. 1927. The effect upon the threshold for nervous excitation of the length of nerve exposed and the angle between current and nerve. Journ. Physiol., 63 : 357.
Rushton W. A. H. 1931. The normal presence of α- and γ-excitabilities in the nerve-muscle complex. Journ. Physiol., 72 : 265.
Rushton W. A. H. 1932. Identification of Lucas's α-excitability. Journ. Physiol., 75 : 445.
Rushton W. A. H. 1934. A physical analysis of the relation between threshold and interpolar length in the electric excitation of medullated nerve. Journ. Physiol., 82 : 332.
Rushton W. A. H. 1935. The time factor in electrical excitation. Biol. Rev., 10 : 1.
Rushton W. 1937. Initiation of propagated disturbance. Proc. Roy. Soc., Ser. B, 124 : 210.
Sacks J. 1944. Radioactive phosphorsstudies on hexosomono-phosphate metabolism in resting muscle. Amer. Journ. Physiol., 142 : 155.
Sacks J. 1948. Mechanism of phosphate transport across cell membranes. Cold. Sp. H. Symp. Q. B., 13 : 180.
Schiodt E. 1931. On the quantity of free water in the red blood corpuscles. Biochem. Journ., 25 : 8.
Schmitt F., R. Bear and E. Ponder. 1938. The red cell envelope considered as a Wiener mixed body. Journ. Cell. Comp. Physiol., 11 : 309.
Schmitt F. O., R. S. Bear and R. H. Silber. 1939. Organic and inorganic electrolytes in lobster nerves. Journ. Cell. Comp. Physiol., 14 : 351.
Schmitt F. O. and O. H. Schmitt. 1940. Partial excitation and variable conduction in the squid giant axon. Journ. Physiol., 98 : 26.
Schoepfle G. M. and Erlanger J. 1941. The action of temperature on the excitability, spike height and configuration and the refractory period observed in the responses of single medullated nerve fibres. Amer. Journ. Physiol., 134 : 694.
Schonheimer R. 1942. The dynamic state of body constituents. Harvard Univ. Monogr. : 1—72.
Schriever H. 1932. Über Einschleichen von Strom: quantitative Verschiedenheiten. Ztschr. Biol., 93 : 123.
Schriever H. und H. Bürkner. 1940. Die elektrische Erregbarkeit des Nerven bei Durchströmung verschiedener langer Strecken. Pflüg. Arch., 243 : 223.
Schriever H. and H. Ehrhardt. 1939. Der Zeitenquotient bei der Narkose des Nerven. Pflüg. Arch., 242 : 730.
Schultze M. 1863. Das Protoplasma der Rhizopoden und der Pflanzenzellen. Ein Beitrag zur Theorie der Zelle. Leipzig : 1—68.
Schulze P. 1927. Über die Grösse des Anteils der interstitiellen Gewebsflüssigkeit am Gesamtquerschnitt des quergestreiften Froschmuskels. Ztschr. exper. Med., 56 : 470.
Scoglund C. R. 1945. The electrotonic changes of excitability in nerve and their relation to accomodation. Kungl. Suenska Vetenskapsakademiens, 21. 9 : 1.
Seelich F. und L. Stockinger. 1953. Ein Beitrag zum Problem der Zellform und deren Umwandlung. Ztschr. Zellforsch., 39 : 212.
Segal J. 1953/54a. Elemente einer Theorie der Nervenerregung. I. Eiweissalteration und Nervenerregung. Wiss. Ztschr. Humb. Univ., 3 : 243.
Segal J. 1953/54b. Elemente einer Theorie der Nervenerregung. II. Polarisationserscheinungen in heterogenen Medien. Wiss. Ztschr. Humb. Univ., 3 : 257.
Segal J. 1956. Bemerkungen zur Theorie der Erregung. Intern. Symp. über d. Mechanismus. d. Erregung. Berlin, 22—24 Nov. 1955 : 14.
Shaw F. H. and S. E. Simon. 1955. The nature of the sodium and potassium balence in nerve and muscle cells. Austral Journ. Exper. Biol. Med. Sci., 33, 2 : 153.

Silber R. H. 1941. The free amino acids of lobster nerve. Journ. Cell. Comp. Physiol., 18 : 21.
Silber R. H. and F. O. Schmitt. 1940. The role of free amino acids in the electrolyte balance of nerve. Journ. Cell. Comp. Physiol., 16 : 247.
Simon A. und J. Szelöczey. 1928. Untersuchungen über Kalium- und Kalziumgehalt der peripheren Nervenfasern. Biochem. Ztschr., 193 : 393.
Sivian L. and S. White. 1933. Minimum audible sound fields. Journ. Acoust. Soc. Amer., 4 : 176.
Slator A. 1906. Studies in fermentation. Part I. The chemical dynamics alcoholic fermentation yeast. Journ. Chem. Soc., 89 : 128.
Slyke Van D. D. and G. M. Meyer. 1913. The fate of protein digestion products in the body. III. The absorption of aminoacids from the blood by the tissues. Journ. Biol. Chem., 16 : 197.
Söhngen N. and K. Wieringa. 1926. Determination of permeability with Saccharomyces cerevisiae. Proc. Roy. Acad. Amsterdam, 29 : 353.
Solandt D. J. 1936a. The measurement of accomodation in nerve. Proc. Roy. Soc., Ser. B, 119 : 355.
Solandt D. J. 1936b. Comparison of various methods of measuring time-constant of accomodation of nerve. Proc. Roy. Soc., Ser. B, 120 : 389.
Solomon A. K. 1952. The permeability of the human erythrocyte to sodium and potassium. Journ. Gen. Physiol., 36 : 57.
Solomon A. K. and G. L. Gold. 1955. Potassium transport in human erythrocytes: evidence for a three compartment system. Journ. Gen. Physiol., 38 : 371.
Stämpfli R. 1952. Bau und Funktion isolierter markhaltiger Nervenfasern. Erg. Physiol. Biochem., 47 : 70.
Steinbach H. B. 1933. Injury potentials in scallop muscles. Journ. Cell. Comp. Physiol., 3 : 203.
Steinbach H. B. 1937. Potassium and chloride in thyone muscle. Journ. Cell. Comp. Physiol., 9 : 429.
Steinbach H. B. 1940a. Sodium and potassium in frog muscle. Journ. Biol. Chem., 133 : 695
Steinbach H. B. 1940b. Electrolyte balance of animal cells. Cold. Sp. H. Symp. Q. B., 8 : 241.
Steinbach H. B. 1941. Chloride in the giant axons of the squid. Journ. Cell. Comp. Physiol., 17 : 57.
Steinbach H. B. 1944. The osmotic behaviour of frog sartorius muscles. Journ. Cell. Comp. Physiol., 24 : 291.
Steinbach H. B. and S. Spiegelman. 1943. The sodium and potassium balance in squid nerve axoplasm. Journ. Cell. Comp. Physiol., 22 : 187.
Steinhardt J. and E. M. Zaiser. 1951a. Masking of acid-binding groups in native horse carbonylhemoglobin. Journ. Biol. Chem., 190 : 197.
Steinhardt J. and E. M. Zaiser. 1951b. Kinetic evidence on the mechanism of the acid denaturation of horse CO hemoglobin. Journ. Amer. Chem. Soc., 73 : 5568.
Stewart D. 1931a. The permeability of the Arbacia egg to nonelectrolytes. Biol. Bull., 60 : 152.
Stewart D. 1931b. The permeability of the Arbacia egg to ammonium salts. Biol. Bull., 60 : 171.
Stewart D. R. and M. H. Jacobs. 1932. The effect of fertilization of the egg of Arbacia and Asterias to ethylene glycol. Journ. Cell. Comp. Physiol., 2 : 275.
Stewart D. R. and M. H. Jacobs. 1936. Further studies on the permeability of the egg of Arbacia punctulata to certain solutes and to water. Journ. Cell. Comp. Physiol., 7 : 333.
Stockinger W. 1927. Bindegewebe der weissen Maus in verschiedenen Lebensaltersstufen, mit besonderer Berücksichtigung der Mastzellen und der Gewebesleukozyten. Ztschr. Zellforsch. mikr. Anat., 6 : 27.
Strohl A. and A. Djourno. 1942. Recherches sur les variations de rheobase en fonction des variations de température en différentes regions du nerf. C. r. Soc. biol., 136 : 125.
Sugi J. 1935. On the seat of the electrical asymmetry of the injury potential of muscle. Japan. Journ. Med. Sci., Biophysics, 3 : 268.
Svedberg A. 1933. On the distribution of sugar between plasma and corpuscles in animal and human blood. Skand. Arch. Physiol., 66 : 113.
Swellengrebel N. 1905. Über Plasmolyse und Turgorregulation der Pressbefe. Bakt. Cbl., II. Abt., 14 : 374, 481.
Szpilman I. und B. Luschinger. 1881. Zur Beziehung von Leitungs- und Erregungsvermögen der Nervenfaser. Pflüg. Arch., 24 ; 347.

Tarver H. and W. O. Reinhardt. 1947. Methionine labeled with radioactive sulphur as an indicator of protein fermentation in the hepatectomized dog. Journ. Biol. Chem., 167 : 395.
Tasaki I. 1939. Electro-saltatory transmission of nerve impulse and effect of narcosis upon nerve fiber. Amer. Journ. Physiol., 127 : 211.
Teorell T. 1935a. Studies on the «diffusion effect» upon ionic distribution. I. Some theoretical considerations. Proc. Acad. Sci. (USA), 21 : 152.
Teorell T. 1935b. An attempt to formulate a quantitative theory of membrane permeability. Proc. Soc. Exper. Biol. Med., 33 : 282.
Teorell T. 1936. A method of studying conditions within diffusion layers. Journ. Biol. Chem., 113 : 735.
Trautwein W., K. Zink und K. Kayser. 1953. Über membran- und Aktionspotentiale einzelner Fasern des Warmblüterskelettmuskels und ihre Veränderungen bei der Ischämie. Pflüg. Arch., 257 : 20.
Ungar G., E. Aschheim, S. Psychoyos and D. Romano. 1957. Reversible changes of protein configuration in stimulated nerve structures. Journ. Gen. Physiol., 40 : 635.
Urano F. 1908. Neue Versuche über die Salze des Muskels. Ztschr. Biol., 50 : 212; 51 : 483.
Ussing H. H. 1947. Interpretation of the exchange of radio-sodium in isolated muscle. Nature, 160 : 262.
Velick S. F. 1949. The interaction of enzymes with small ions. Journ. Phys. Colloid. Chem., 53 : 135.
Verworn M. 1896a. Untersuchungen über die polare Erregung der lebendigen Substanz durch den constanten Strom. III. Pflüg. Arch., 62 : 415.
Verworn M. 1896b. Die polare Erregung der lebendigen Substanz durch den constanten Strom. IV. Pflüg. Arch., 65 : 47.
Verworn M. 1914. Erregung und Lähmung. Eine allgemeine Physiologie der Reizwirkung. Jena : 1—304.
Verzar F. 1911. Über die Natur der Thermoströme des Nerven. Pflüg. Arch., 143 : 252.
Volta A. 1803. Fortgesetzte Versuche über die Electrocität. Ann. Physik, 14 : 257.
Vries H. de. 1884. Eine Methode zur Analyse der Turgokraft. Jahrb. wiss. Bot., 14 : 427.
Vries H. de. 1885. Plasmolytische Studien über die Wand der Wacuolen. Jahrb. wiss. Bot., 16 : 465.
Vries H. de. 1888. Osmotische Versuche mit lebenden Membranen. Ztschr. physik. Chem., 2 : 415.
Walker E. 1925. A colour reaction for disulphides. Biochem. Journ., 19 : 1082.
Waller A. D. 1899. The characteristic of nerve. Its alteration by altered temperature. Journ. Physiol., 24, Proc. I—III.
Walter H. 1923. Protoplasma- und Membranquellung bei Plasmolyse. Jahrb. wiss. Bot., 62 : 145.
Warburg O. 1928. Über die katalytischen Wirkungen der lebendigen Substanz. Berlin : 1—528.
Webb D. A. and J. Z. Young. 1940. Electrolyte content and action potential of the giant nerve fibres of Loligo. Journ. Physiol., 98 : 299.
Weber L. und F. Lederer. 1936. Einfluss von ein- und mehrwertigen Alkoholen, sowie von Mono- und Polysacchariden auf die Flüssigkeitsaufnahme durch Gel. Biochem. Ztschr., 285 : 115.
Weidmann S. 1951. Electrical characteristics of Sepia axons. Arch. Ital. biol., 114 : 372.
Weiss M. G. 1901a. Recherches sur l'excitation des nerfs par les courants de très courte durée. C. r. Soc. biol., 53 : 253.
Weiss M. G. 1901b. Excitation du nerf par deux ondes électriques successives et très courtes. C. r. Soc. biol., 53 : 400.
Weiss M. G. 1901c. La loi de l'excitation électrique des nerfs. C. r. Soc. biol., 53 : 466.
Weiss M. G. 1901d. Sur la possibilité de rendre comparables entre eux les appareils servants à l'excitation électrique. Arch. Ital. biol., 35 : 413.
Wilbrandt W. 1938a. Die Permeabilität der Zelle. Ergebn. Physiol. exper. Pharmakol., 40 : 204.
Wilbrandt W. 1938b. Die Permeabilität der roten Blutkörperchen für einfache Zucker. Pflüg. Arch., 241 : 302.
Wilde W. 1945. The chloride equilibrium in muscle. Amer. Journ. Physiol., 143 : 666.

W i l l i a m s R. and G. K a y. 1945. Absorption, distribution and excretion of thiourea. Amer. Journ. Physiol., 143 : 715.
W i n t e r s t e i n H. 1926. Die Narkose. Berlin : 1—474.
W o o d E., D. C o l l i n s and G. M o s. 1940. Electrolyte and water exchanges between mammalian muscle and blood in relation to activity. Amer. Journ. Physiol., 128 : 635.
W o o d b u r y J. W. 1952. Direct membrane resting and action potentials from single myelinated nerve fibers. Journ. Cell. Comp. Physiol., 39 : 323.
W o o d b u r y L. A., H. H. H e c h t and A. R. C h r i s t o p h e r s o n. 1951. Membrane resting and action potentials of single cardiac muscle fibers of the frog ventricle. Amer. Journ. Physiol., 164 : 307.
W o o d b u r y L. A., J. W. W o o d b u r y and H. H. H e c h t. 1950. Membrane resting and action potentials of single cardiac muscle fibres. Circulation, 1 : 264.
W o o d h a u s D. and F. P i c k w o r t h. 1932. Permeability of vital membranes. XXXV. The red blood corpuscles. Biochem. Journ., 26 : 309.
W u H. 1922. Separate analysis of the corpuscles and the plasma. Journ. Biol. Chem., 51 : 21.
W u H. and E. F. Y a n g. 1931. Permeability of muscle to sodium ion. Proc. Soc. Exper. Biol. Med., 29 : 248.
Y o u n g A. C. 1938. The effect of stimulation on the potassium content of Limulus leg nerves. Journ. Neurophysiol., 1 : 4.

AUTHOR INDEX

NOTE: The numerals refer to the page numbers of the Russian original, which appear in the left-hand margins of the translation.

Russian Authors

Abrikosov, I. A. 289, 302
Afanas'ev, P. V. 135
Aizenberg, E. I. 11, 113-115, 117-120
Aleksandrov, V. Ya. 11-13, 15-17, 19, 20, 24, 27, 31, 32, 49, 53, 55, 62, 71-73, 80, 90, 104, 150, 158, 159, 169, 177, 180, 184, 185, 187, 193-198, 206, 210, 211
Aleksandrov, S. N. 51, 52, 191
Anokhin, P. K. 295
Arronet, N. I. 80
Averbakh, M. S. 224-236, 239, 240, 255, 263-272, 355, 360, 365;
see also Ushakov et al. 355, 365-368.
Babkin, B. P. 95
Barbashova, Z. L. 12, 208, 209
Barkhash, A. P. 136
Belitser, V. A. 17, 25, 28
Beritov, I. S. 305, 347
Biderman 263
Blokhina, V. P. 24, 25
Boldyrev, V. B. 85, 92
Boldyreva, N. 145
Borzdyko, S. 205
Braun, A. A. 11, 12, 27
Braun, A. D. 21-24, 83
Brusilovskaya, A. L. 125
Burlakova, V. 153
Butkevich, V. P. see Troshina, V. P.
Chagovets, V. Yu. 185, 186, 279, 286, 335, 338
Chakhotin, S. 50
Chepinoga, O. L. 156
Cherepanova, T. N. 70, 103, 365;
see also Ushakov et al. 355, 365-368
Chetverikov, G. N. 86, 93
Chir'ev, S. 334, 339
Danzhar, P. 169
Darkshevich, V. N. 289, 302
Demin, N. N. 136
Dolidze, Sh. V. 152
Dontsova, Z. N. 314, 317
Dorfman, V. 113
Dzhamusova, T. A. 56, 65, 69, 74, 206, 272-274
Engel'gardt, V. A. 122, 123, 211
Epshtein, Ya. A. 152
Fedorova, E. A. 295
Fel'dman, N. L. 21, 32
Filippova, V. N. 286, 287, 291, 301
Frank, G. M. 90
Frenkel' Ya. I. 40
Fridlyand, I. B. 136
Gabrichevskii, G. 262
Gavrilova, L. 59, 66
Gel'khorn, E. 122, 130, 138
Genin, M. M. 87, 94
Ginetsinskii, A. G. 12, 208, 209
Golikov, N. V. 306, 313
Golovina, N. V. 42-47, 67, 85, 88, 102, 103, 210
Gramenitskii, E. M. 191, 192
Grindel', O. M. 240
Gryunkhagen 263
Gulinova, E. N. 263
Gur'eva, I. P. 136
Gzhatskii, S. Z. 124
Il'in, V. S. 148
Il'ina, Yu. N. 135
Il'inskaya, N. B. 56
Indzhikyan, A. T. 263
Ivanov, M. F. 11, 12, 27
Kamnev, I. E. 11, 12, 14, 124
Kan, I. L. 88, 109
Kaplanskii, S. Ya. 143, 145
Karaev, A. I. 206
Karaev, A. O. 214, 235
Karasik, V. M. 272
Kasatochkin, V. I. 48
Kazmina, N. A. 48
Khlopin, N. G. 100
Khodorov, B. I. 263, 264, 355, 361-368
Kiro, M. B. 53, 61, 62, 86, 87, 93, 103
Kiselev, P. A. 289, 302
Klein, E. E. 152
Kolotilova, A. I. 122, 123
Komarova, G. 224, 272
Kometiani, P. A. 152, 209, 210
Komkova, A. I. 18
Kondrashova, M. N. 78, 79
Konikov, A. L. 307, 312, 313
Konikova, A. S. 138
Kornakova, E. V. 90
Korsakova, G. F. 87
Koshtoyants, Kh. S. 26, 159, 207
Kotlyarevskaya, M. A. 85, 92
Krasil'nikova, V. L. 32
Krokhina, E. M. 92
Krolenko, S. A. 56, 350, 351, 373, 376
Kulakova, A. F. 136
Kupalov, P. S. 151, 186
Kursanov, A. L. 134, 174

Kuznetsov, D. F. 88
Kvasov, D. G. 70, 218, 263, 274
Lazarev, N. V. 41, 125
Lebedinskii, A. V. 206, 207
Lepeshkin, V. V. 106, 167, 168
Leushina, L. I. 191
Lev, A. A. 85, 91, 375
Levin, S. V. 86, 92, 93, 101, 102
Lopatina, N. G. 56, 59
Lozina-Lozinskii, A. L. 57
Lyudkovskaya, R. G. 90
Magnitskii, A. N. 300, 306
Maiorchik, V. S. 295
Makarov, P. V. 11, 12, 15, 55, 92, 99, 169
Makarov, P. O. 199, 200, 215, 247-249, 254, 261
Maksimov, N. A. 110, 111
Meshcherskaya, K. A. 11, 12, 119
Mikhailov, A. N. 168
Mikhel'son, N. I. 206
Miropol'skii, S. V. 136
Misheneva, V. S. 80-82
Momzen, 263
Morozova, R. I. 21
Mozhaeva, G. N. 375
Mozhaeva, L. V. 110
Mozzhukhin, A. S. 206
Muzheev, V. A. 205, 300
Nasonov, D. N. 11-13, 15-17, 19, 20, 24, 27, 31-40, 49, 51-55, 58-64, 66-68, 78, 85, 87, 89, 90, 95, 96, 98, 99, 104, 113-115, 119, 120, 150, 158, 159, 169, 177, 180, 184, 185, 187, 191, 193, 198, 206, 210, 220-222, 224-236, 238-240, 242, 243, 247, 250-253, 255, 257, 260, 261, 264-272, 280, 282, 284-287, 289, 290, 293, 297-300, 302, 307-313, 315-325, 327-330, 337, 339, 342, 345, 348, 353
Naumenko, A. L. 218, 263
Navakatikyan, A. O. 289, 302
Nikol'skii, N. N. 375
Nusel'man, E. I. 125
Oparin, A. I. 119, 169, 174, 175, 183
Panteleeva, N. S. 74, 77, 78
Pasynskii, A. G. 24, 25, 27
Pavlovskaya, T. E. 24
Pelishchenko, I. A. 144
Pisareva, L. N. 151, 153-155
Ponomarenko, V. V. 65, 272-274
Raevskaya, M. A. 11, 12, 30, 51, 191, 192, 195, 199
Ravdonik, K. S. 33-38, 40, 53
Rezvyakov, N. P. 246, 248, 263
Romanov, S. N. 30, 37, 85, 86, 91-93, 101-103
Romanova 192
Rozental', D. L. 38, 39, 51-54, 57-59, 66, 85, 91, 92, 191, 220, 222, 236, 238, 242, 243, 247, 250-253, 255, 257, 260, 261, 280, 282, 284-287, 289-291, 293, 297, 298, 300-302, 307-313, 316, 337, 348, 353, 375,
Rubashkin, V. N. 95
Rubinshtein, D. L. 108, 111, 122, 133, 147, 201, 203, 209, 280
Rusinov, V. S. 70, 240
Saichuk, V. I. 136
Savich, V. V. 95
Savost'yanova, M. V. 21
Sechenov, I. M. 306
Semiotrocheva, N. L. 110
Shapiro, E. A. 56, 59, 86, 93, 348, 375
Shcherbakov, B. I. 110
Shteingauz, L. N. 90
Shtutina, B. L. 100
Sisakyan, N. M. 175
Slavutskii, Ya. L. 295
Smitten, N. A. 85, 89, 91
Smoilovskaya, E. 83
Soreni, E. T. 156
Speranskaya, T. A. 210
Stepanova, S. S. 92
Strel'tsova, S. V. 88
Subbotin, M. 262
Suzdal'skaya, I. P. 12, 46, 47, 54, 58-64, 66-68, 78, 85, 87, 89, 90, 96, 98, 99, 103, 224, 240, 264, 298, 299, 315-325, 327-332, 365; see also Ushakov, et al. 355, 365-368
Szent Gyorgyi, A. 152, 159, 204
Tarusov, B. N. 153, 210
Tikhal'skaya, V. V. 148
Tongur, A. M. 48
Tongur, V. S. 47, 48
Trifonova, A. N. 12
Troshin, A. S. 12, 29, 30, 104, 112, 119, 120, 124-127, 129-137, 139-143, 150, 151, 153, 156, 160-166, 168-171, 173, 180, 183, 202, 204
Troshina (Butkevich) V. P. 67, 68, 365; see also Ushakov et al. 355, 365-368
Uflyand, Yu. M. 286, 289, 291, 302, 306
Ukhtomskii, A. A. 70, 215, 236, 240, 248
Urinson, A. P. 144
Ushakov, B. P. 18, 56, 57, 59, 70, 74, 85, 88, 89, 91, 206, 355, 365-368
Ushinskaya, O. F. 263, 274
Vandokanti, F. I. 124
Vasil'ev, L. L. 113
Vereshchagin, S. M. 87, 94
Verigo, B. F. 218, 242, 247-249, 263, 353, 355-362, 365, 367-369
Verkhovskaya, I. 133
Vladimirov, G. E. 144
Vorob'ev, V. E. 124
Volkova, M. S. 24, 25
Vorontsov, D. 246, 248
Vul, I. M. 307, 312, 313
Vvedenskii, N. E. 11, 14, 70, 74, 103, 212, 214, 215, 224, 236, 237, 240, 242, 244, 248, 262, 263, 276, 277, 300, 304, 370, 372

Yampol'skaya, M.N. 136
Yasvein, G.V. 72
Yudenich, N.A. 263
Yurkevich, V.V. 119
Zarakovskii, G.M. 85, 86, 101, 102
Zbarskii, B.L 135, 136
Zelenkova, N.P. 60, 66
Zhinkin, L.N. 87
Zhirmunskaya, E.A. 88
Zhirmunskii, A.V. 87, 102
Zhukov, E.K. 53, 70, 78, 88, 314, 317
Zubkova, L.R. 136

Foreign Authors

Abelson, P.H. 163
Adler, E. 148
Adrian, E.D. 213, 261
Alderson, E. 106
Amson, K. 145, 147
Anson, M.Z. 17
Apostolaki, J. 299
Arnett, V. 158
Arnold, V. 26
Aschheim, E. 90
Asmussen, E. 220
Aten, A. 156
Auger, D. 218
Azuma, R. 303
Bale, W. 144, 146, 148
Barnes, T.C. 207
Basset, J. 47
Bear, R.S. 107, 136, 149, 151
Bedo, M. see Hétenyi, Issèkutz et al. 124
Berglund, H.B. 123, 130, 138
Bernstein, J. 179, 186, 188–191, 196, 200, 201
Berwick, M.C. 153
Beutner, R. 179, 182, 207
Bialaszewicz, K. 113
Bishop, G. 226, 230, 234, 235
Blair, E.A. 218, 295, 361
Blair, H.A. 314, 317
Bloor, W.R. 158
Blumenthal, A. 263
Borscok, H. 138
Boruttau, H. 246, 247
Bowditch, H.P. 212
Boyle, P.J. 147, 148, 156
Bridgman, P.W. 41, 47
Bronk, J.K. 47
Brooks, S.C. 122, 156
Brown, D.E.S. 42, 47
Brücke, E. Th. 213
Brunswick, D. 248, 249, 261, 262
Brutkowski, S. 275
Bugnard, L. 337
Bullock, T.H. 207, 245, 254, 260
Bungenberg de Jong, H.G. 167–170, 183
Burdon-Sanderson, J. 200, 201
Burge, W.E. 189
Bürkner, H. 340, 349
Campbell, D.H. 47
Cardot, H. 303, 340, 343, 344
Cattel, McK. 41, 42, 46
Chambers, A.H. 155
Chambers, E.L. 156
Chambers, L.A. 33, 39
Chambers, R. 50, 110
Christiansen, H. 136
Christopherson, A.R. see Woodbury, L.A. et al. 202
Chu, C.H.U. 147
Cobb, D. 143, 145–147, 149, 151, 156–158, 161, 162, 165, 175
Cohn, E.F. 144
Cohn, W.E. 144
Cole, K.S. 202, 286, 353
Colle, J. 286, 314, 353
Collins, D. 158
Conway, E.J. 130, 147, 148, 156
Cowan, S.L. 158
Cremer, M. 184, 295, 305
Csúcs, L. 158
Cuffler, S.W. 53
Curtis, H.J. 202
Daniel, E.E. see Fing, et al., 201
Danielli, J. 107, 203
Davis, H. 248, 249, 261, 262, 303, 306
Davson, H. 144
Dean, R.B. 145, 147
Deasy, C.L. 138
Dendrinos, G. 242, 246
Deriand, R. 299
Dervichian, D.G. 168, 169
Deuticke, H.I. 47, 75–78
Di-Moia, F. 23, 26
Djourno, A. 315
Dokan, Sh. 107
Dow, R.B. 47
Draper, M.H. 202
DuBois-Reymond, E. 216, 278, 279, 356, 359, 360
Dubuisson, M. 153
Duclaux, J. 169
Duryee, W.R. 163
Dziemian, A.J. 107
Ebbecke, U. 41, 42, 46, 47, 67, 75–78, 279, 286 335, 338
Edwards, D.J. 42

Efron, J. 244
Ege, R. 113, 122, 123, 129
Eggleton, M. 124
Eggleton, Ph. 131
Ehrhardt, H. 301
Eichler, O. 156
Eichler, W. 340
Elliott, G.C. 156
Ellsworth, R. 107
Embden, G. 148
Engelmann, T. 190–192, 200, 279
Ephrussi, B. 113
Erlanger, J. 218, 226, 230, 234, 235, 295, 315, 319, 361
Ernst, E. 152, 156–158, 204
Eyring, H. 47
Fabre, 125
Fahr, G. 156
Fredet, 125
Fenn, W.O. 108, 131, 143, 145–149, 151, 153, 155–158, 161, 162, 165, 166, 175, 186, 187, 189, 190
Feynn, F. 145
Fick, A. 88, 279
Fing, E. 201
Fischer, A. 24
Fischer, M. 106, 167
Flaig, J.V. 90
Fleischmann, W. 122
Flick, J. 145
Flosdorf, E.W. 33, 39
Folin, O. 123, 130, 131, 135
Fontaine, M. 42
Forbes, A. 248, 249, 261, 262, 306, 313
Frédéricq, H. 48
Fricke, H. 107
Fricker, J. 152, 158, 204
Fröhlich, F.W. 242, 244, 246–249
Fujita, A. 107
Furchgott, R.F. 148, 156
Furusawa, K. 205, 207
Gad, I. 244
Gallison, W.E. 152
Garcia Ramos, J. 212, 214, 216
Garten, S. 196
Gáspár-Németh, Zs. see Hetényi, Issèkutz et al. 124
Gasser, H.S. 53, 54, 74, 75, 226, 230, 234, 235
Gaunt, W.E. 148
Gerard, R.W. 80, 201, 205, 207
Gold, G.L. 145
Gotch, F. 200, 201, 314, 334
Graham, J. 201
Granit, R. 361
Greese, R. 146
Griffith, H.D. 148
Grijns, G. 122, 130, 135, 144
Grundfest, H. 202, 207
Guilliermond, A. 169
Gutstein, M. 138
Haagen-Smit, A.J. 138
Haege, L. 145, 147, 153, 158, 165, 166, 175
Hahn, L. 144, 145, 153, 156
Hamburger, H.J. 130, 144
Hamilton, P.B. 136, 147
Harris, E.J. 145
Hartline, H.K. 113
Harvey, E.N. 47, 107, 203
Hasenbring, O. 42, 67
Hastings, A.B. 144, 156.
Haurowitz, F. 23, 25, 26
Häusler, H. 129
Hecht, H.H. see Woodbury et al. 201, 202
Hedin, S.G. 122, 130, 144
Heffter, A. 26
Hegnauer, A. 143, 149, 151, 156, 157, 162, 165
Heilbrunn, L. 147
Heinbecker, P. 248
Henderson, P. 184
Heppel, L.A. 146, 158
Hermann, L. 187–189, 196, 216–218, 335, 338, 356, 369
Hertz, H. 204
Hess, A. 147
Hetényi, G. 124
Hevesy, G.C. 144, 145, 148, 153, 156
Hill, A.V. 112, 124, 130, 148, 151, 186, 263, 279, 285, 286, 335, 337, 338, 354, 355, 360, 361, 366
Hill, S.E. 218
Hiruma, K. 135
Höber, R. 106–108, 124, 130, 135, 136, 138, 144, 145, 147, 148, 179, 180, 182, 203
Hodgkin, A.L. 143, 147, 150, 155, 158, 173, 176–178, 201, 202, 213–215, 218–221, 223, 225, 228, 235, 236, 240, 241, 252, 253, 275, 349
Hoffman, C. 122
Hoffmann, F. 107, 130
Höfler, K. 110
Holböll, S. 130
Holleman, L. 169, 170
Hoorweg, I.L. 279–287, 289, 295, 303–306, 308, 335–338, 353, 354
Hopkins, A. 248, 249, 261, 262
Huber, B. 110
Huxley, A.F. 158, 201, 202, 219
Irving, J.T. 148
Irving, L. 143
Issèkutz, B. 124
Jacobs, M.H. 113
Jasper, H.H. 306, 309
Jinnaka, S. 303
Johnson, F.H. 47
Juni, E. 156
Kamen, M.D. 145, 156
Kamer, van der J.C. 315
Kane, F. 130, 147
Kato, G. 213, 242, 247–249, 261, 262

Katz, B. 202, 213, 214, 218, 235, 241, 263, 264
Kay, G. 130
Kayser, K. 201
Keighley, G. 138
Keil, F. 340
Keth, N.M. 123
Keynes, R.D. 146, 147, 149, 155, 158, 177
Klotz, I.M. 156
Knake, Ch. 72
Koeppe, H. 144
Korr, I.M. 209
Kozawa, Sh. 122, 135, 144
Kramer, B. 151
Krigsman, B.J. 315
Krogh, A. 109, 124, 143, 145, 147, 173, 176, 177
Kronfeld, P. 210
Krouse, R. 189
Kruyt, H. 167, 168
Kühne, W. 72, 74, 105
Kunitz, M. 17
Lambert, E.T. 306, 313
Lanczos, A. 248
Lapieque, L. 281, 285–287, 289, 294, 295, 297, 303–307, 309, 312–314, 317, 335, 337, 338, 354
Lapicque, M. 306, 308, 314, 315
Larrabee, M. 113
Laufler, M.A. 47
Laugier, H. 303, 340, 343, 344
Lederer, F. 119
Lennep, van E.W. 315
Leskinen, V. 300
Lewis, P.R. 149, 155
Lichteneckert, J. see Hétenyi, Issèkutz u.a. 124
Lillie, R.S. 217
Lindberg, A.L. 124, 146
Lindberg, O. 156
Lodholz, E. 246
Loeb, J. 263
Lorente, de No R. 213
Lowy, P.H. 138
Lucas, K. 213, 243, 246, 247, 261, 287, 295, 314
Lucké, B. 113
Luco, J.V. 212, 214, 216
Lund, E.J. 209
Lundegardh, H. 108, 109, 173, 176, 178
Lundsgaard, C. 130
Lundsgaard, E. 74
Luschinger, B. 242, 246, 247
Lynch, E. 136
Macdonald, J.S. 314, 334
Macheboeuf, M.A. 47
Maisel, G.W. 147
Maizels, M. 145
Manery, J.F. 143, 144, 146, 148, 158
Mangold, E. 48
Marcuse, A.J. 339
Marsh, B. 143, 146, 147, 149, 151, 156, 157, 161, 162, 165, 175
Masing, E. 122, 123, 129
Marsland, D.A. 47
Matthews, J. 47
Matula, I. 188, 189, 196
Mayer, A. 263
McCutcheon, M. 113
Meyer, G.M. 136
Meyer, K.I. 108
Michaelis, L. 107, 108, 179, 210
Mines, G. 314
Mirsky, A.E. 17, 18
Modderman, R.T. 169, 170
Möllendorff, M. 72
Möllendorff, W. 72, 74
Mond, R. 122, 130, 145, 147, 158
Monnier, A.M. 306, 309
Moore, B. 106
Moore, G. 106
Mos, G. 158
Mueller, C.B. 144, 156
Mullins, L.J. 145, 152, 153, 156
Nachmansohn, D. 207–209
Nastuk, W.L. 202
Nathansohn, A. 107
Nernst, W. 179, 181, 184, 279, 280, 284–286, 305, 335–339, 346, 352–354, 361
Netter, H. 128, 149, 158
Neukomm, A. 113
Neuschlosz, S.M. 152
Noonan, T.R. 145, 153, 158
Oker-Blom, M. 189
O'Reilly, H.L. 147
Ørskov, S.L. 113
Osterhout, W.J.V. 218
Ostwald, W. 179
Ottesen, J. 148
Overton E. 105–107, 111–115, 117, 124, 130, 135
Page, J.H. 113, 151
Paine, S. 133
Parker, E. 107
Parpart, A. 130
Parviainen, S. 300
Pauli, W. 188, 189, 196
Perlzweig, W. 107
Pfeffer, W. 105, 110–112
Pflüger, E. 354–356, 358, 360, 361, 363, 365, 367, 368
Pickworth, F. 123, 129, 130
Pond, S.E. 218, 219
Ponder, E. 107, 112
Porter, R.R. 26
Power, M.H. 123
Prankerd, T.A.J. 145
Prosser, C.L. 155
Psychoyos, S. 90
Pumphrey, R.J. 213, 241
Putnam, F.W. 25, 26, 28
Quincke, G. 106, 107
Rebbe, O. 144, 148

Reginster, A. 152, 158, 204
Reinhardt, W.O. 138
Renquist, J. 300
Rényi, G. 50
Ricca, R. 113
Ritchie, A.D. 213
Roaf, H.E. 106
Romano, D. 90
Roncato, A. 130
Ronzoni, E. 80
Rosenberg, H. 337, 340, 348
Rosenberg, Th. 109, 173, 176, 178
Rosenblueth, A. 212–214, 216, 239
Rotschub, K.E. 52
Rothenberg, M. 207
Ruhland, W. 107
Ruiter, L. 169
Rushton, W.A.H. 202, 213, 215, 236, 287, 33[illegible], 340, 347–349, 351
Sacks, J. 156
Saint Hilaire, C.C. 262
Sandor, C. 47
Schaefer, H. 42
Schaffer, L. 156, 158, 204
Scheminsky, F. 210
Schindt, E. 113
Schmeiser, K. 156
Schmidt-Nielsen, B. 146
Schmitt, F.O. 107, 136, 149, 151, 221, 235, 241
Schmitt, O.H. 213, 218, 221, 235, 241
Schoepfle, G.M. 315, 319
Schonheimer, R. 138
Schriever, H. 301, 340, 349
Schultze, M. 105
Schulze, P. 124
Scoglund, C.R. 361
Seelich, F. 72
Segal, J. 221
Shaw, F.H. 150
Shorr, E. 148, 156
Sheridan, E. 145
Shull, J. 130
Sievers, I. 108
Silber, R.H. 136
Simon, A. 152
Simon, S.E. 150
Skinner, B.F. 306, 313
Slator, A. 135
Slyke, van D.D. 136
Söhngen, N. 133
Solandt, D.Y. 263, 264, 366
Solomon, A.K. 145
Spiegelman, S. 145, 149, 156
Stämpfli, R. 202, 217, 219, 334
Steinbach, H.B. 147, 149, 153, 156, 163, 164, 175, 189
Steinhardt, J. 26
Stewart, D.R. 113
Stockinger, W. 72
Stolk, A. 315
Strohl, A. 315
Suet, W. 106, 167
Sugi, J. 189, 196
Svedberg, A. 123, 130, 131
Swellengrebel, N. 133
Szeloczey, J. 152
Szpilman, I. 242, 246, 247
Takacs, I. 156
Tarver, H. 138
Tasaki, I. 218, 248
Tekman, S. 23, 26
Teorell, T. 108
Tisdall, F. 151
Trautwein, W. 201
Trelles, R.A. 152
Tschermak, A. 196
Tupikova, N. 80
Turner, R.S. 245, 254, 260
Ungar, G. 90
Urano, F. 156
Urquhart, J.M. 156
Ussing, H.H. 146
Velick, S.F. 156
Verworn, M. 50, 82, 246, 261
Verzar, F. 188
Volta, A. 278
Vries, H. de 105, 110–112
Walker, E. 26
Waller, A.D. 314, 317
Walter, H. 110, 111
Walter, K. 152
Warburg, O. 82, 211
Webb, D.A. 149
Weber, L. 119
Webster, I. 106
Weech, A. 107
Weidmann, S. 202
Weiss, M.G. 279, 280, 282–285, 295, 305, 306, 335–338, 353
White, S. 40
White, W.E. 156
Wiame, J.M. 156
Wieringa, K. 133
Wilbrandt, W. 108, 109, 130, 173, 176, 178
Wilde, W.S. 147, 153, 158
Williams, R.W. 47
Winter, M. see Hetényi, Issèkutz et al. 124
Winterstein, H. 82
Wood, E. 158
Woodbury, J.W. 201
Woodbury, L.A. 201, 202
Woodhaus, D. 123, 129, 130
Wright, G.G. 47
Wu, H. 123, 130, 131, 135, 145
Yamatori, A. 107
Yang, E.F. 146
Young, A.C. 158
Young, J.Z. 149, 213, 241
Zaiser, E.N. 26
Zink, K. 201

ACID, ACETIC, effect of, on the pancreas of the mouse 97
ACID HYDROCHLORIC, effect of, on the pancrease of the mouse 97
increase in muscle excitability 273
threshold concentration of, for the narcotization of muscles 66
ACETYLCHOLINE 207
ACCOMMODATION 263, 336, 354, 355, 358, 360, 366
according to the ideas of Verigo 263, 361
as cathodic depression 263, 361
according to Nernst 355, 361
velocity of 264, 366
changes in, by calcium chloride 264, 366
by the action of chemical agents 366
ACCOMMODOMETRY 361
ADAPTATION, in receptors 274
cellular 58, 59
threshold of (during narcosis) 59, 61
ADENOSINE diphosphate 79, 80
ADENOSINE triphosphoric acid 78, 79
ADENYLPYROPHOSPHATE 189
of nerve, during block 81
ADRENALIN, threshold concentration for contraction of fibrocytes 73
ADSORPTION, by coacervate micelles 169, 171
of protoplasm 125, 129, 134, 160, 163, 180
on the surface of particles 122, 127
nonpolar, Langmuir curve 128, 134
constant "a" of Langmuir equation 128
limit A_∞ in Langmuir equation 128
graphical method of determination of 128
for muscles, different substances 174
ADSORPTION CURVE 129
ALANINE, time necessary for the establishment of diffusion equilibrium of, in muscles 136, 137
effect of , on the volume of muscle 115
the constant A_∞ of, for muscles 137, 174
the distribution coefficient K of, for muscles 137
molecular weight of 119
penetration of, into muscles 136, 137
killed by alcohol 136, 137
ALBUMIN, serum 18, 24, 25
egg 18, 24
effect of, on the volume of muscles 115, 117
molecular weight of 118
ALCOHOL, isoamyl, effect on mouse pancreas 97
narcosis, paranecrosis, stimulation of muscle 66
distribution between the simple coacervate and equilibrium fluid 169
ethyl, effect on mouse pancreas 97, 99
as a narcotic for the nerve 243, 255, 297
narcosis of muscles 60, 61
of the nerve 297
threshold concentration for cellular narcosis 61
contraction of fibrocytes 73
ALKALI, narcosis, paranecrosis and stimulation of muscles 66
ARABINOSE, distribution of, between muscles and medium 129
adsorption limit A_∞ of, in muscles 174
AMINOACIDS, coefficient of distribution Q of, for muscles 136
erythrocytes 136
permeation into the cell, of 135, 138
investigation of, by the use of labeled atoms 138
distribution of, between the cell and the medium 135, 137
content of, in nerve fibers of cephalopod mollusks 136
AMMONIA (as a narcotic for the nerve) 243
ANIONS, deficiency of, in the protoplasm 151
penetration of, into the cell 144
into erythrocytes 144
Heber, series of, according to the degree of the positive charge of the cell surface 180
ARABINOSE, distribution of, between muscles and medium 129
adsorption limit A_∞ of, in muscles 174
ASPHYXIA, effect of, on living cells 12, 15, 206
AUTOMATIC ACTIVITY, during increase of excitability 262
BARIUM CHLORIDE, narcosis, paranecrosis, and stimulation of muscles 66
BISECTRIX of the coordinate angle 161, 171, 221, 238
BLOCK of a nerve by heat 81
by cold 82
of the nerve fiber (nonconductivity) 81, 219
BRAIN, mouse, stainability under hydrostatic pressure 46
preparation of, in the living animal 92
BRAIN CORTEX of the rat, stainability upon a conditioned reflex excitation 93
of the mouse, stainability upon stimulation 92, 93
BRAIN TISSUE of rats, excess of cations in 152
BRIDGE, salt 218
CAFFEINE 79
CATELECTROTONUS, 357, 359, 361, 366
CATHODE, effect of, on living cells 15
of polarizing current, change of nerve excitability under, 354
"CATHODIC PEAK" (local contraction of muscles under the cathode) 42
CATIONS, prevalence over anions in the protoplasm 151
permeation of, into erythrocytes 145
the Heber series of, according to the degree of

---

* [See note to Author Index.]

negativity of the cell surface 180
CALCIUM, excretion by muscles into a medium devoid of calcium 157
arrest of the multiple response of the nerve to constant current, by 263
binding by protoplasm proteins 152
chloride, effect on the pancreas of the mouse 97
narcosis, paranecrosis and stimulation of muscles by 66
threshold concentration for the contraction of fibrocytes 73
CELL, yeast, permeability of 133
change in volume of 113, 172
as an osmometer 109, 111, 113, 138, 144, 339
as an electric condenser 279, 338
of boundary tissues, secretory, or resorbing 177
vital staining of, during excitation, similarity to the histological preparation 13
permeability of, to nonelectrolytes 119, 159
of plants, nonvacuolated 110, 133
the osmotic pressure of the protoplasmic sap and the vacuole sap 110
of plants, permeation of substances, into 110, 132
pH shift upon excitation 13
secretion, stimulation of 95, 97
from tissues of the internal environment of the organism 177
death of, exit of potassium and phosphates 143
penetration of sodium and chlorine into 143
CELL COLLOIDS, decrease in the degree of dispersion upon excitation 12
CESIUM CHLORIDE, narcosis, paranecrosis and stimulation of muscles 66
CILIA, movement of 224
CHLORALHYDRATE, narcosis, paranecrosis and stimulation of muscles 66
CHLORINE, distribution coefficient K in muscles 161, 175
between the protoplasm and the medium 161
penetration into cells 13, 167
into muscles of the squid 177
muscle fibers 147, 158, 161
distribution between yeasts and medium 160
concentration in the gigantic nerve fibers of the Loligo 149
of nerve fibers 147
CHLOROFORM as a narcotic for the nerve 82, 243
CHOLINESTERASE 207
CHROMATIN, grains of, staining upon excitation of the cell 13
CHRONAXIMETRY 291
CHRONAXY 281, 287, 333
graphic determination of (from the intensity-time curve 296
dependence of the value of on the interelectrode distance 303, 343, 344
relationship to rheobase 304
change due to the action of alcohol 297
as a measure of the threshold time of excitation 296, 306
rate of reacting (as a time factor) 294, 295, 297, 298, 301, 302, 306
ratio of a/b 295, 296, 299, 304, 308, 326, 343
of frog muscles, dependence of the value on size of electrode 303
in human subjects (in health and disease) 301
of the nerve following sectioning the latter 298, 306
in rats 298, 310
changes with time, in vaseline 326
in frogs, changes following treatment with alcohol 297, 299
of various organisms 304
overlapping with the point of intersection of the asymptotes of the intensity-time curve 305
CHONDRIOSOMES of pancreas cells 98, 99
distribution of, in the cell 177
COACERVATE COMPLEX 168, 169
effect of salts on the potentials of 183
of gelatin and gum arabic with the addition of hydrochloric acid 169
distribution coefficient Q (between the coacervate and the liquid at equilibrium) 169
formation of surface films consisting of orientated particles 169
separation of vacuoles 169
decrease in solubility of 171
simple 168
distribution of substances between the coacervate and the medium, similarity to living cells 169-171
bound water of the hydration layer 168, 171
water content of 168
composition of as compared with protoplasm 168, 169
equation of the distribution curve of substances 171
COCAINE, effect of on the rheobase and chronaxy of the frog nerve 300
COLD, effect on the concentration of potassium and sodium in the nerve fiber 177
CONDENSOR time of discharge 287
CONDUCTION of potential, monophasic 226
CONSTANT 'a', units of measuring of 281, 284, 289
dependence on the histological structure of the tissue 292, 303
method of measuring 303
with changes in temperature 319
as the threshold time of excitation 281, 293, 306
short-term threshold of excitability 281, 291, 292, 293, 316, 343
in human muscles 301
in rat muscles, dependence on temperature 332
in frog muscles, dependence on temperature 331
in pigeon nerves, dependence on temperature 329
in rat nerves 298, 309, 311, 327
dependence on temperature, of 327, 329
change with time, of, in vaseline oil 326
in frogs 297, 300, 308, 316, 318, 322, 323
changes in, by the action of alcohol 290, 297
determination of, (experimental) 289
upon sectioning the nerve 299, 309

dimensions of 289, 292, 316
seasonal changes of its dependence on temperature 322
'b', protracted excitability threshold (rheobase) 280, 289, 291, 292, 343
dependence of, on the histological structure of the tissue 292
on temperature 319
in human muscles 301
in rat muscles, dependence on temperature 332
in frog muscles, dependence on temperature 331
in muscles of the leech 294
in pigeon nerves, dependence on temperature 329
in rat nerves, 298, 309, 311, 327
dependence on temperature 327, 329
changes with time of, in vaseline oil 326
of frog nerve, 294, 297, 300, 308, 316, 318, 322, 323
change of, by the action of alcohol 290, 297
upon cutting the nerve 299
seasonal changes of temperature dependence of 322
'n', graphic determination of 288
of the human biceps 287
of the frog stomach 293, 294
of the frog muscle 294
of nerves and muscles of vertebrates 300
definition of 285
range of values of (0.5-1.0) 284, 286, 305, 339, 353
errors of graphical determination of 284
upon the action of various agents 289
of the sciatic nerve of the frog 308
of the frog heart 294
stability of, during changes in intensity-time curves 333
the incline factor of 283, 285, 293, 333
the physiological meaning of 285
CONSTANTS 'a' and 'b', independence of changes in 296, 333
the formulas of Weiss 279
of Hoorweg 279, 283
of Hoorweg-Weiss 280
of excitability 281, 333
CONTRACTION 53, 55, 74
idopathic 70
as a local stable excitation 54, 62, 70, 74
cathodic 70, 71
of muscle 42, 52-54, 62, 64, 70, 73, 78
of the acetylcholine type 53
veratrine-like 53
nonspecific 53, 78
metabolism of 74, 75, 78
following action of acetylcholine 78, 94
of hydrostatic pressure 67, 75, 77, 78
of metabolic inhibitors 206
of potassium chloride 65
of urea 78
CONTRACTION of muscle following the action of sodium chloride 64, 65, 273
after previous denervation 65, 273
high temperature 67
hydrochloric acid 68
chloral hydrate 78
ethyl alcohol 63
ether 63, 64
during maintenance in Ringer's solution 74
specificity of, following the action of various agents 71
CONTRACTION of cells (non-muscular), quantitative matter of determination 72, 73
local, dependence on voltage of stimulating current 220
of fibrocytes, as local excitation 74
following the action of adrenalin 73
potassium chloride 73
calcium chloride 73
novocaine 72, 73
alcohol 73
gradual 73
trypan blue 73
in Ringer's solution 73
excitation 72
rate of 74
ether 63, 64
upon maintenance in Ringer's solution 74
specificity of, upon the action of various agents 71
CORTI'S ORGAN 32, 33
as amplifier of sound 41
CREATINE, excretion of by the cells during excitation 13
by muscles during excitation 83
by testes, during excitation 83
CREATINE PHOSPHATE 79, 80, 189
of muscle during contraction 75, 78
during tetanus 75
during tonus-like contraction 78
resynthesis of 76
in the nerve during a block 81
CREATININE, distribution coefficient K for muscle 132
Q for blood 131
for muscle 131
adsorption limit A_∞ in muscle 132, 174
CRESOL as a phase 182
CRINOMA, 100
"CURRENTS OF ACTION", see Potential of action
"CURRENTS OF REST", see Potential of injury
CURVE of dependence of local reaction on intensity of stimulus 214
of the velocity of cellular narcosis on the concentration of the agent 56
canonic, of Hoorweg 308
absorption of aqueous solutions of dyes 21
of salt solutions of dyes 21
of threshold energy, minimal 339
of distribution of a substance between the cell and the medium 126, 160

according to the law of Henry 127
intensity-time of the biceps of the human arm 301
intensity-time, logarithmic form 282, 283, 288
of muscles of the hamstring group in man 301
of the frog stomach 294
of the rat, effect of temperature on 332
of the frog, intersection of, with effect of temperature 331
of pigeon nerves 298, 330, 331
change of, upon cooling 298
of the rat 298, 328, 331
change of, upon cooling 298
of the frog 298, 317, 318, 322, 353
intersection of, upon change of temperature 317, 318, 322, 329
seasonality of 322
of the tortoise, intersection of, upon change in temperature 324
of nerves of coldblooded animals, intersection
of following effect of temperature 325
divergence of, for different organisms 285
complete equation of 284
in different shunts 288
different organisms 284, 294, 336, 339
of the frog heart, intersection following effect of temperature 314
agreement with the formula (of Nasonov) 286
tangent of slope of left-hand branch 284
S-shaped, of gradual dependences 220, 277
of the dependence of protein alteration on pH 221
of the magnitude of response on the intensity of excitation 221, 276, 278, 375
for single fibers 375
in various functional states of the conducting fiber 238, 376
CYANIDES, effect on a concentration of potassium and sodium in nerve fibers 177
CYSTEIN, blocking of SH-groups 27
CYTOPLASM, staining with vital stains 13
sorption of the stain during excitation 13, 138
DAHLIA VIOLET, increase of excitability of muscles, by 273
DAMAGE, irradiation of, in cells of nonconducting tissues 50, 52
in protoplasm 50
of muscles, irradiation, dependence on the thickness of the fiber 51
on the electroconductance of the medium 51
the electric mechanism of spreading of 51, 219
DECREASE of tissue stainability after excitation 101
DECREMENT, area in cm during above-peak excitation 234
of conduction, apparent, upon narcotization of the nerve, 242, 260
during narcosis 246, 248
of the spreading impulse in the nerve of Anodonta 88
DENATURATION of proteins, see Proteins, denaturation of
DEPRESSION, cathodic, of Verigo 353, 355-359, 361, 363
DEXTRIN, effect on the volume of muscle, of 115, 117
of egg cells of marine animals 117
molecular weight of 119
DIFFUSION, rate of 21, 132
across membranes 112
DINITROPHENOL (2,4-) 79
effect on the potassium and sodium content of the nerve fiber, of 177
DISACCHARIDES, effect on the volume of egg cells of marine animals, of 117
permeation into erythrocytes, of 122
DISTRIBUTION coefficient Q of substances (adsorbed plus soluble) 123, 126, 128, 130, 169
K 128, 130, 161, 175
between the surrounding water and the water of the protoplasm 128, 167
graphic representation of 128
of sugars 129
"EFFECTIVE TIME" 305
EGGS of sea urchins, accumulation of phosphates in 156
prevalents of cations 151
egg cell of marine animals, bound water content of 113
release of sodium when damaged 163
ELECTRO-ADSORPTION 141
ELECTRODE differential 291
ELECTRODES, calomel 264
capillary 303
short circuit of 347
nonpolarizing 185, 362
(DuBois-Reymond), their resistance 343
polarization on 342, 343, 344
excitatory, destroying the current loops (on the oscillograms) 255
recording (location) 226
ELECTROLYTES, "assymetry" of distribution between cell and medium 143, 151, 172
return into the protoplasm of the resting cell 150, 158
exit from isolated tissues 156
change in concentration on the electrodes 335
of the cell, adsorbed or chemically bound 166
ionized, dissolved in water of the protoplasm 166
firmly bound with the protoplasm 166
three different states of 166
direction of diffusion 167
distribution between cells and medium 160, 166
dependence on concentration in medium 166
solubility in protoplasm 166
bond with protoplasm proteins 151, 180
rate of penetration into cells 166
content of muscle sap 187
condition of in the protoplasm 150, 166, 167, 172

ELECTRONARCOSIS as cathodic parabiosis 240
ELECTROTONUS, physiological 263, 355, 356
of Pflüger 354, 355, 365
physical 355, 356
ENERGY of stimulating current, of stabilization 288, 316, 319, 352
of current, value of 352
ENZYMES, activity, dependence on substrate concentration 134
dependence on soluble or bound state 175
EPITHELIUM, ciliary, metabolism of 80
of frog kidney, vital staining during action of audible sounds 38
EQUATION of Henry 128, 129, 175
for distribution of substances between coacervate and medium 171
of Langmuir for nonpolar absorption 128
of Nernst 346
ERROR of measurement, mean square of 34, 234, 318
ERYTHROCYTES, exit of potassium during hemolysis 153
irradiation of damage 50
of the cat, prevalence of sodium over potassium 153
distribution coefficient K for galactose 129
Q of glucose 123
of dextrose 123
of urea 123
Q of fermentable sugars 123
of the rabbit, amount of bound water 112
impermeability to glucose, sodium chloride and sodium sulphate 113
metabolism of potassium in a potassium medium 153
labeled potassium of the medium, rate of 145
sodium on a potassium medium 144
labeled sodium of the medium (rate of) 144
adsorption limit of the sugar 171
permeation of cations 145
sugars during arrest of glycolysis 123
permeability to electrolytes 144
concentration of sugars in various animals 123
ESERINE, effect on the potential of action 207
damage of muscles 208
suppression of cholinesterase 207, 208
ETHER, ethyl, effect on mouse pancreas 97
as a narcotic for the nerve 243
EXALTATION PHASE 84
(of excitability) of Vvedenskii 237
increase in excitability 244
EXCITABILITY, effect of a volley of impulses originating at the site of sectioning the nerve 312
of smooth and striated muscles 294
graphic illustration of (S-shaped curve) 238, 252, 278
biphasicity of change during narcosis 300
protracted excitability threshold, see Constant 'b'
dependence of, on duration of current pulses 356
on interelectrode distance 339
according to Rushton 348, 351
on threshold energy of stimulating current 350
change in region of anode of polarizing current 355, 358, 364, 365
of cathode of polarizing current 354, 355, 358, 362, 366
at poles, on breaking the current 356
under cathode of polarizing current, dependence on temperature 364
upon narcotization 241, 242, 244, 246, 300
measurement of 277
as a magnitude reciprocal to the threshold 278
quantitative evaluation of (by the square of the threshold voltage) 352
constants of 277, 333
short-term threshold, see Constant 'a'
maximum of, within physiologically adequate time intervals 294
of muscles in the absence of calcium 224
of limbs in man 301
of the cat, following effect of chemical agents 79
increase of, by the action of glycerol 263
by drying 263
independence from temperature (in region of physiologically adequate durations of stimulation), of 319, 330, 331
of the nerve, in vaseline oil 315, 316, 326
effect of temperature on 314
of the rat, changes in, after separation from the central nervous system 310, 311
maximum of (according to threshold energy) 353
upon the action of temperature, treated with monoiodoacetate 323
seasonal changes 322
heating 318
cooling 317
drying and swelling 315
decrease in, caused by calcium 263
of the tortoise, during the action of temperature 324
of a single nerve fiber, independence on temperature of 315
of nerve centers 370
evaluation of, by threshold voltage of excitatory current 352
by threshold of spreading impulse 277
increase of, in the vicinity of a cut nerve 263
transition into nondecremental propagation 225
into rhythmic activity 225, 262, 264, 265, 270, 273, 276
after preliminary stimulation 45, 102
EXCITATION, nondecremental spread of 221
appearance of, at the cathode 335, 355, 368, 369
gradual, dependence of on intensity of the stimulus 221, 237
decremental, spread of 221, 239
dependence of, on the voltage of the stimulating current 344
on the intensity of the stimulating current 344, 352

on the energy of the stimulating current 319, 334, 344, 346, 347, 351, 352
on the amount of electricity 334, 347
on the velocity of conduction on the electroconductivity of the medium 218
incremental spread of 221
of the cell 49, 95, 371, 373
excretion of potassium 163, 173, 205
excretion of phosphates 173
as a cyclic process 374
reversible, release of bound electrolytes 176
definition of the term 48, 371
the membrane theory of, see Theory of Excitation, membrane
local, value of, according to the area on the oscillogram 227
gradual dependence of, on the intensity of the stimulus 213, 229, 275, 276, 277
and its spread, general signs 212, 240, 372
similarity of physicochemical changes in the protoplasm, in 372
transition into a spreading one 213, 225
spread with decrement, of 214, 241
stable or parabiosis 212, 240
mechanism of appearance of isolated impulses 369
negativity of the stimulated sector of the conducting fiber 372
a solitary wave of 369
spreading 50, 239, 371
gradual theory of, see Theory of Excitation, gradual
law of autoregulation, see Law of autoregulation of spreading excitation
connection with local reaction 212, 225
similarities with parabiosis 240
S-shaped curve of dependence of magnitude of response of the conducting tissue on intensity of the electric stimulus, see Curve, S-shaped
increase in electroconductivity 205
phenomenon of summation 214
increase of upon cutting the nerve 307
upon passage of impulses 313, 314
of the heart under influence of narcotics 224
EXCITATION of the cell, reversibility of 13
threshold 28
above-peak potential 225
unipolar 291
FIBROBLASTS in cultures, contraction by the action of agents 72
FIBROCYTES, contraction upon excitation 72
effect of agents on 72, 73
FIXATION of cells with osmic acid 92, 99
according to Ciampi 97
FLUORODINITROBENZINE (1:2:4), NB, 26
FORMALIN, preservation of the phase property of the protoplasm after fixation of cells in 210
FORMULA of Weiss 279
of Nernst, for threshold current intensity 279, 280, 284, 335, 354
for phase potentials 182
of Hodgkin and Katz, for calculating the value of the potential of rest 202
of Hoorweg 279, 280, 289, 304, 336, 354
of Hoorweg-Weiss 280, 282-284, 295, 305, 335, 353
of Schriever and Burkner 349
GALACTOSE, effect on the volume of muscle, of 115
dependence of the distribution coefficient of, on the concentration in the medium 126
distribution coefficient K of, for erythrocytes 129
molecular weight of 119
limit of adsorption A_{∞} of, in muscles 174
permeation into muscles 129, 174
into erythrocytes 119, 126, 129
distribution of, in the complex coacervate 169
between the muscles and the medium 129
of erythrocytes, adsorbed 129
dissolved 127
GANGLIA of mammals, stainability on excitation, biphasicity of 102
sympathetic, stainability under the influence of audible sounds 37
upon stimulation 88
spinal, vital staining of 38
stainability after cessation of excitation 92, 101
of the rabbit, stainability upon stimulation (shock) 91
under the influence of audible sounds 37
of the rat, stainability during excitation 92
by a conditioned reflex 93
of the frog, stainability upon excitation 90, 91
dependence of, on the initial functional state 92
stainability upon the action of audible sounds (of maximal frequency) 38
GELATIN, dehydration by nonelectrolytes, of 119
GLANDS, stimulation, increase of sorption of a vital stain 97
GLAND, salivary, of the larva of the mosquito Chyronomus 100
of the mouse, stainability under pressure 46
GLOBULIN, serum 18, 26
of egg white 18
GLOBULIN X, bond with potassium 152
GLUCOSE, effect on the volume of muscle 115
molecular weight of 119
permeation into the cell, of 178
rate of fermentation, of 135
GLYCEROL, effect on the rhythmic activity of nerves 268
on the volume of muscle 115
of egg cells of marine animals 117
molecular weight of 119
GLYCOGEN 75, 76
of muscle, upon contraction 75
GLYCOLYSIS 80, 123
GOLGI APPARATUS, polar location of, in the cell 177

GRANULE formation in cells 13, 32, 100
in muscles 32, 139
change of, upon excitation (biphasic) 13
as complex coacervation 32
GRANULES of dye in the living cell 13, 27, 32, 83, 141
deposition of in the cell, reversibility of 100
in the loops of the Golgi apparatus 32, 95, 100
of secretion, stainability of 97
GRADIENT of diffusion of a narcotic 242, 249, 255, 259, 261
concentration 121, 146, 148, 150, 158, 1 9, 164, 167
GUAIACOL as a phase 182
GUM ARABIC, effect on the volume of muscles, of 115, 117
molecular weight of 118
HEAT resistance of tissues, dependence on the heat resistance of their proteins 18
HEART, excitability of the automatic regulation center of 224
effect of narcotics on 224
of frog, excitability with changes in temperature 314
HENRY, law of, see Law of Henry
HEPARIN 24, 25
HEMOGLOBIN, homodromic reversibility of denaturation by sodium salicylate 17
HIGH-ENERGY phosphorus-containing compounds 75
HYDROCHLORIC ACID, effect of on pancreas of mice 97
increase of muscle excitability by 273
threshold concentration of, for narcotization of muscles 66
HYDROGEN CHLORIDE 79
HYPERBOLIC dimensions (or the second constant of the hyperbola) of Renquist, Leskinen and Parviainen 300
HYPERTONICITY, effect on living cells, of 12
on the pancreas of mice 98
HYPOTONICITY, effect on living cells, of 12, 83
INCREMENT 214, 276
of the spreading impulse after emerging from the narcotized nerve segment 252
INHIBITORS of metabolism 57, 206
nonspecific effect of 206
poisoning of the cell by 177
INVERTASE, activity of 135
IMPEDENCE of cells, upon stimulation 204
IMPULSES, a volley of, from traumatized area 312
centrifugal 275
IMPULSE, spreading, value of the peak of, in a narcotized segment, graphic determination of 249, 256
decrement upon narcotization 242, 244, 247, 249, 254, 261
in above-peak excitation 229, 234, 236, 276
dispersion of velocities in the whole nerve 234
distance of run with increment 235
duration of the rising peak 302
dependence of the value on the site of the above-peak excitation 234
on the electroconductance of the medium 218
incremental run 223, 225, 235, 252, 276
mechanism of conduction along the fiber 217, 249, 276
relation of the value of, to the value of the threshold impulse, see Safety Factor
regulation of the value of the peak, protective value of 276
IMPULSE, solitary, origin of 370
determination of the value of, at the site of, or in the vicinity of the site of excitation 226
jump of, to neighboring fibers 218
across a blocked segment 218
spreading 48, 213
automatic regulation of the magnitude of 223, 225, 235, 236, 276
nondecremental conduction of 244, 251, 261
value of, graphical presentation (S-shaped curve) 221, 278
determination of, from the area 226, 2 8
INHIBITION, cellular 374
IRRITABILITY 11, 15
appearance of, in the process of evolution 15
KIDNEY of mammals, phosphate metabolism, in state of hypotonicity 83
LABELED atoms in studies of permeability 162
LABILITY of tissues 224, 300
as a characteristic of the velocity of processes 277
LACTIC ACID 75, 77
LACTIC ACID, of the muscle, during contraction 75
of the nerve, in a block 81
LACTOGLOBULIN (β) 26
LACTOSE, effect of, on the volume of muscles 115
in yeast cells, adsorption by the protoplasm 134
solubility constant K, of 134
adsorption limit A_{∞} of, 134
molecular weight of 119
permeation of, into yeast cells, dependence on the concentration in the medium 133
LATENT PERIOD 366
of paranecrosis 17
LAW of "all or none" 77, 212, 214, 215, 229, 236, 237, 247, 275
for nerve and muscle fibers 213, 275, 373
of the heart 213
of Henry 127, 129, 132, 141, 155, 160, 163, 166
of an isolated conduction of an impulse 218
of constancy of threshold energy (of current) 354
of excitation (DuBois-Reymond) 278
of autoregulation of spreading excitation 237
LIMIT A_{∞} in the Langmuir equation 128
graphic method of determination of 128
for muscles, series of substances 174

series of substances for muscles 129, 174
LITHIUM CHLORIDE, narcosis, paranecrosis and stimulation of muscles 66
LIVER of mammals, phosphate metabolism under hypotonic conditions 83
LOOP of polarizing current (interaction with the testing current at the electrodes) 356, 358
of excitatory current, earth circuit 226, 230, 255, 266
bridge for reducing of 230
negative 227
positive 226
LUCIFERASE, effect of pressure on 47
MACRONUCLEUS of certain parasitic infusoria, vital staining of 13
MACROPHAGES 72
MAGNESIUM, distribution coefficient K of, in muscles 166, 175
distribution curve, between muscles and medium 166
binding by protoplasm proteins 152
chloride, muscle narcosis 57
paranecrosis and stimulation of muscles 66
MECHANONARCOSIS of Ebbecke 42, 47
MEMBRANE, cellular, selective "pumping in" of substances 150, 151
selective permeability of 145
impermeability to anions 144, 148, 186
permeability to ions 143, 186, 189, 201
semipermeable, at the surface of cells 105, 107, 109, 120, 167, 172, 180, 279, 338, 339
METABOLISM of nonconducting tissues 80
of nerve upon stimulation 80
basal, of the cell 177
of phosphorus compounds of muscles during contraction 75, 77
in tetanus 77
in tonus 77
METHIONINE, labeled 25, 27, 138
MITOCHONDRIA 178
MONOIODOACETATE, effect on nerve excitability of, as a function of temperature 323
potential of muscle damage 205
of the muscle 206
MONOSACCHARIDES, effect of, on the volume of egg cells of marine animals 117
permeation of, into erythrocytes 122
MUSCLE, excitability after decompression 45
in the absence of calcium 273
stimulation, reversible decrease in volume 158
decrease in the amount of bound potassium 158
release of vital stains 139
MUSCLE, release of potassium in an isotonic solution devoid of potassium 156
upon stimulation 158, 163
of calcium, into a medium devoid of calcium 157
of phosphates 148
of Tentholdea; potassium content of 164
dependence of the stainability of, on hydrostatic pressure, biphasicity 102
exchange with potassium from the medium 146, 154
sodium from the medium 146, 154
excess of cations in 151
irradiation of damage in striated ones 51
dependence on the presence of calcium 51
of insect appendages 191
velocity of 51, 195, 199
in the lobster 191
electric mechanism of 51
amount of bound and free potassium in 152
contractions of, classification 53
under pressure 75
by the action of sodium chloride 64, 65
distribution coefficient of sugars in 129
of the frog, effect of temperature upon excitability of 331
excitability of 273
denervation of 65
change in volume of 113, 115
anion and cation content of 143
killed, stainability 140
under pressure 43
under the influence of audible sounds 34
distribution of alanine (A_{∞} and K) in 137
of Limulus 218
of mammals, denervated, stainability upon acetylcholine contraction 94
swelling in hypotonic solutions 112
narcosis 56, 57, 58, 59, 60
metabolism during stimulation 80
stainability by neutral red diluted with distilled water 138
under pressure 102
under the effect of sound of maximal frequency 35
of audible sounds 33, 34
denervation, biphasicity 102
$\frac{Na}{Cl}$ ratio 146
damage 51
action potential, value of 200
potential of injury, value of 197, 199, 200
loss of water in hypotonic solutions 112
adsorption limit A_{∞} of alanine 137, 174
arabinose 129, 174
galactose 129, 174
potassium 153, 174
creatinine 132, 174
magnesium 174
sodium 174
saccharose 129, 174
phenol red 174, 141
penetration of sugars in 170
permeability to ions 146
to nonelectrolytes 132
dimensions of the intercellular space 147
distribution of sodium and chlorine between muscles and medium 161

of dyes between muscles and medium 139
rhythmic activity upon increase in excitability 36, 273
heart, ion exchange in 146
decrease in injury potential after sectioning 190, 193
damage, lack of irradiation of 192
skeletal, of the frog, irradiation of damage 191
decrease in potential after sectioning 190, 193
intensification of stainability upon stimulation 93
water content in living 154
potassium content of 163
in three fractions (states) 154, 163
sodium content of 154
in three fractions 154
contraction of, upon the action of audible sounds 36
dependence of, on intensity (decibel) 37
upon increase of hydrostatic pressure 42
metabolism, upon stimulation 157
human, determination of excitability of 291, 301
threshold of electric stimulation 279
embryonic, acetylcholine contraction of 94
MUSCLE fiber, single (of the tetanic bundle of m. ileo-fibularis) of the frog 94, 350
S-shaped curve of (gradual dependence) 375
permeability of 147
to electrolytes 145
MUSCLE sap, electrolyte content of 187
MYOALBUMIN, bound with potassium of 152
MYOGEN, bond with potassium of 152
MYOSIN, gel, binding of potassium, by native and denatured ones 152, 153
of rabbit, temperature of denaturation 18
of frog, temperature of denaturation 18
splitting off of potassium, upon denaturation of 152
bond with potassium, of 152, 204
calcium 152
magnesium 152
enzymatic properties of 211
MYOSIN fibers, denaturation of 22
binding of dyes by 22
NARCOSIS of a fiber, changes in excitability, graphic representation of (S-shaped curve) 240, 244
increase in threshold of 240, 297
phases of 241, 244, 249, 253, 255, 256
recovery from, phase of increased excitability 244
biphasicity of change in excitability 300
decrement of conduction in single nerve fiber 260
cellular, graduality of 55
dependence of time of onset on concentration of the agent (formula) 57
as reversible loss of excitability 55
of muscles, upon the action of inhibitors of metabolism 57
of potassium chloride 65
reversibility of 65
magnesium chloride 57
sodium chloride 64
elevated temperatures 67
hydrochloric acid 66
ethyl alcohol 60, 61
ether 63
reversibility 63
increase of survival time, in subthreshold concentrations of the agent 61
threshold concentration of the agent 57, 65
of muscle 31, 55
as refractivity of local stable excitation 70
definition of 70
of nerve, biochemical shifts in 81, 82
restoration of nondecremental conduction, development with time 259
by the action of high temperature 248
of mechanical pressure 248, 261
decrement of impulse conduction by 247, 248, 249
development with time 255, 256
total 242, 255, 259
carbohydrate-phosphorus metabolism 81
determination of depth of narcosis from the time of maintenance of conduction through the segment 254
graphic method of (S-shaped curve) 254
conduction, according to scheme of Davis, Forbes, Brunswick and Hopkins 262
conduction, according to scheme of Kato, 248, 249, 262
decrease in amplitude of spreading impulse 259
NARCOTIZATION of nerve, dependence of time on length of the segment 248, 249, 261
stage of absolute refractivity 241, 244
of relative refractivity 241, 244
of increased excitability 244
NARCOTIC, effect on living cells 15
reversibility of paranecrosis 15
penetration into erythrocytes 125
NECROSIS, as indication of irreversible injury to tissue 14
NERVE of Anodonta, decrement in staining upon excitation 88, 89
threshold of 279
nonmedullated, of the crab Hyas coarctatus 349
of Maja 340, 348
excitability of, changes in a moist chamber, under the influence of temperature 315
stimulation of, release of potassium 158
propagation of spreading impulses 234
change in volume in solutions of salts, acids and alkalis 113
measurements of excitability, direct (from the value of the local electric reaction) 277
isolated, release of electrolytes by 156
of the crab, increased staining affinity upon stimulation 88, 89
of the rat, change in excitability with immersion in vaseline oil 326
intensity-time curves of 298, 299

reversibility of temperature changes of excitability 327
of the frog, release of electrolytes 156
anion and cation content of 143
local electric reaction 213
multiple response upon closing and breaking of circuit of constant current 263
medullated, enhancement of stainability upon stimulation 88, 89
untiring of 276
metabolism upon stimulation 80
of the lake frog Rana ridibunda 341
prevalence of cations over anions 151
injury potential in the moist chamber and in Ringer's solution 193
decrease in, after cutting 190, 193, 194
value of action potential exceeding that of injury potential 200, 201
rhythmic activity on increase in excitability 265
sciatic of the frog, excitability constants 287
resistance of, to various agents 352
of the tortoise (Emys orbicularis) 324
NERVE branches, terminal 273
motor endings of frog muscles, staining affinity during excitation 93
NERVE fiber of invertebrates, decremental spread of excitation in 222
giant, of cephalopod mollusks, effect of metabolic inhibitors on 177
permeability of to electrolytes 149
of the squid, dependence of the velocity of impulse conduction on the electroconductance of the medium 219
excess of cations in 151
ion content of 149
of mollusks, survival in vitro 155
single, of Panulirus 245
of the earthworm (Lumbriculus terrestris) 245
of the squid, decrement of impulse 235
injury potential and action potential 203
of the worm Marphysa 245
of the crab, survival in vitro 155
conduction of excitation 219, 252
single, excitability, independence of temperature 315
decrement of conduction during narcosis 254, 260
of the squid, S-shaped curve of dependence of response on excitation 221, 375
of the crab Carcinus 349
Hyas araneus 272
rhythmic activity of 272
velocity of impulse conduction in sea-water 219
in paraffin oil 219
damage of, lack of irradiation 192
NEURON 274
NONELECTROLYTES, dehydration curves for living and killed muscles 116, 119
penetration into the cell 119, 121, 122, 132
distribution between cell and medium 134, 141, 160
NOVOCAINE, effect of, on vital staining of tissues 72
threshold concentration for contraction of fibrocytes 72
NUCLEOLUS, changes in staining upon excitation 13
NUCLEUS of fibrocytes rounding of during contraction of cells 74
lack of structure when viewed in dark field before action of stimulus 12
staining with vital stains 13, 27
appearance of structures upon excitation 12, 92
membrane 13
ORTHOPHOSPHATE, penetration into cells of 156
OSCILLOGRAM, determination of the value of the spreading impulse 228
sorption of dye upon excitation 13, 138
PANCREAS of mice, stimulation (depletion) upon excitation 96, 97
stainability of, under pressure 46
of fed mice, intensification of staining of 96, 97
PARABIOSIS, analogy to the refractivity of propagating excitation 240
as a local reaction 11, 14, 74, 214
local stable excitation 79, 212
stationary excitation wave 240
of the muscle 70
of the cat muscle, metabolism during 78
phases of 78, 79
of the nerve, signs of 240
prodromal stage of 103
hyperexcitation, stage of 103
PARANECROSIS 11, 14, 17-19, 27, 49, 52, 55, 61, 100, 174, 212
graduality of 27, 216
of gland cells of the pancreas 99, 100
as cellular excitation 49
as local excitation 84
reversible alteration 49, 71
reversible damage 49, 71
reversible condition of the cell during excitation 14, 15, 371
reaction of living matter to external influences 14, 15, 49
stimulus of biochemical reactions 84
of pancreas cells upon the action of novocaine 72
quantitative method of colorimetric determination of 27
of nerve cells, upon the action of novocaine 72
of fibroblasts upon the action of novocaine 72
of the epithelium of kidney tubuli, upon the action of novocaine 72
nonspecificity of the protoplasm response to various agents 15
of the mouse pancreas, excretion of proxymogen granules 99
in asphyxia 15, 206

upon the action of physiologically adequate stimuli 90
signs of 84
specificity of action of agents (stimuli) 15
PARTHENOGENESIS (artificial), as cell stimulation 48
PENDULUM of Helmholtz 362
PERMEABILITY, cellular 174, 372
osmotic method of study of 105, 121, 125, 144
direct method of study 121, 122, 126, 144
PERMEABILITY THEORY, see Theory of Permeability
PHASE of decrease in staining affinity 103
PHENAMINE 79
PHENOL, effect on the rheobase and chronaxy of the frog nerve 300
as a phase 182
PHENOL RED, coefficient of distribution in muscles K 175
absorption limit A_{00} 174
PHOSPHATE, return into the protoplasm of a resting cell 14, 173
exit from cells 13, 148, 158, 173
of nonconducting tissues of mammals during hypotonia 83
of muscles, metabolism during contraction 78
inorganic, penetration into heart muscle of the dog 148
metabolism during parabiosis 79
relation to proteins of the protoplasm 158
PHOSPHOGEN in spermatozoa 80
PHOSPHOCREATINE of muscle upon contraction 75
PHOSPHORUS, concentration in muscle 155
inorganic of muscles during contraction 77
of the nerve during a block 81
PLASMA, blood, $\frac{Na}{Cl}$ ratio in 146
PLASMOLYSIS of plant cells 110, 111, 132
POLYSACCHARIDES, penetration into erythrocytes 122
POTASSIUM in muscles, exchange with potassium from the medium 153
rate of 155
3 fractions of 154, 155, 163, 164
in crab and lobster nerves 166
of a frog, seasonal dependence 165
of erythrocytes of different animals, exchange for the potassium of the medium 145, 153
re-entry into the protoplasm of the resting cell 14, 158, 173
exit from cells 13, 144, 163, 173
from muscles upon stimulation 163
velocity of 146
from the nerve 158
diffusion of, into cuttle-fish fibers, rate of 150
iodide, distribution of, between a simple coacervate and the liquid at equilibrium 169
concentration of in the cell 163, 164
distribution coefficient K of, in muscles 163, 175
distribution curve, for muscles 163
for the nerve 165
exchange with sodium in erythrocytes 144
permeation of, into muscle fibers 146
into Tenthoidea muscles 147, 154
into erythrocytes 144
binding of, by protoplasm proteins 152, 158, 163, 211
content of, in the giant nerve fibers of the squid 149
chemical bond with the protoplasm 151
chloride, effect of, on the pancreas of the mouse 97
enhancement of excitability of muscles by 273
threshold concentration of, for cellular narcosis (of muscles) 65
for contraction of fibrocytes 73
POTENTIAL, bioelectric, the dependence of on the metabolism of the cell 205, 207
of action, increase with increment 222, 225
local, decremental spreading 235
exceeding in value the potential of damage 200, 201
autoregulation of the constancy of the peak magnitude 222, 225
diffusion 183, 198
during stimulation of the cell 186
role of, in the formation of salt potentials 184
a cation series 185
formula of the value of emf 184
concentration 210
local, value of (determined by the area) 228
exceeding in value the spreading potential 229, 237
of above-peak excitations 229
of a muscle mince 187
redox 209
intracellular 210
of infusoria 210
of injury, value of, the formula of Hodgkin and Katz 202
effect of salts on the negativity of the site of cutting of muscle 189
of electrolytes 189
dependence on the washing out of electrolytes from the wound 193, 195, 199
on the method of cutting 199
(or current of rest) 51, 184
as a salt potential 185
site of origin (of the jump) in the cell 185, 187, 188, 189, 195
of the muscle, rate of appearance of 196
effect of temperature on 67, 188
initial increase in 196, 197, 199
upon cutting from both ends 198
fixed in formalin 210
of a nerve, effect of temperature on 188
decrease with time 190, 191, 193
in anoxic state of the nerve 205
following effect of metabolic inhibitors 205

increase of, upon renewal of cut 190, 193, 194, 196
subthreshold, graduality of 224
spread with decrement 224
sharp threshold, for transition into spreading impulse 224
threshold, dependence on the interelectrode distance 349
above-peak 225, 373
decremental spread of 225
jump of, at the interphase border 181
salt 180, 183
of the muscle 183
effect of salts on 183
of the coacervate surface 183
absolute value of 183
effect of salts on 183
Heber series of ions 180
phase of Nernst 181, 183
the emf equation of Nernst 182
PRESS, hydraulic 42
PRESSURE, hydrostatic, effect on the mouse brain, of 46
on the intensity of luminescence of bacteria 47
on the muscle of the frog 42-46, 66
on stainability of the protoplasm, biphasicity of 102
on the pancreas of the mouse 46, 98, 99
on the protoplasm 16, 33, 41, 46
on salivary glands of the mouse 46
on functions of the organism and the cell, biphasicity of 44
methods of studying its effect on tissues 43
PROSTIGMIN, effect on muscles, of 208
inhibition of cholinesterase 207, 208
interstitial space, of the muscle "inulin space" 147, 154
"magnesium" 147
"sodium" 147, 154
"sulfate" 155
"chlorine" 147
in the nerve, "sodium" 149, 163
"chlorine" 149, 162
PROTEIN-SALT COMPLEX, decomposition during denaturation of 211
PROTEINS, agents causing denaturation of 16
effect of, on the absorption spectrum of stain solutions ("effect of correction") 21
denaturation of, by acetone 20
the graduality of, 26, 28
change in the configuration of the molecules 25
covalent bond with dyes 22
by urea, homodromic reversibility 17
disturbance of the process of coacervation of the dye 32
nonspecificity of 16
reversibility of 17, 18, 48
release of sulfhydryl groups of 23
increase in the sorptional properties of 16, 19, 21, 25
appearance of amino-groups 26
refringence of 25
disulfide groups of 26
indole groups of 26
carboxyl groups of 26
sulfhydryl groups of 26
phenol groups of 26
under the influence of hydrostatic pressure 47
features of, in vitro (similarity to paranecrosis) 16
by x-rays 24
binding of heparin by, 24, 25
of dyes, by 15, 16, 21, 22, 26, 27, 32
of labeled methionine, by 24, 25
by sonic waves 33
by heat 18-20, 22, 24
by ultraviolet irradiation 20, 24
by ethyl alcohol 20, 24
of the muscle, change of solubility of, during contraction 77
during tetanus 77
native, a series according to their affinities for dyes 22
increase in stability of 16
penetration into the cell, of 119
of the protoplasm, isolated, bond with potassium 152
denaturation upon stimulation 90
bond with cations 152
sorbtion of vital stains by, 141
binding of dye by, quantitative determination of 19, 22
of the triphenyl methane group 22
of horse blood serum 19-21
PROTOPLASM, re-entry of electrolytes into the resting cell 14, 150
exit of water from 119
of ions, upon stimulation 13
as a complex coacervate 169, 172, 173, 178, 180, 372
as a simple aqueous solution 106, 143, 186
as a phase 167, 173, 180, 183, 339
micelles, adsorption of substances, by 141, 163
immiscibility with the surrounding water 105
increase of resistance after excitation 101
of stability 16
loss of phase properties, upon damage and stimulation 159, 167, 176, 185, 186
solubility of ions in the protoplasm water 159
solubility of the permeating substance 127, 141
reaction to external influences 11, 12, 15, 28, 84
bond with electrolytes, of 152, 339
sorption of dyes upon excitation, biphasicity of 102
capacity of denaturation 16
increase of viscosity upon excitation 90
sorption of dyes upon stimulation 16, 21, 27, 34, 85, 90, 92, 94, 96
upon dying 142

increase of sorptional properties of proteins upon excitation 27
phase properties, loss of, upon damage and stimulation 180
PROTRACTED threshold, see Constant 'b'
PROZYMOGEN 96, 97, 98
QUININE HYDROCHLORIDE, effect on narcosis, paranecrosis and stimulation of muscles 66
RADIATION, ultraviolet or ionizing, reversibility of paranecrosis 15
RADIOACTIVITY, specific, method of determination of 153
REACTION, local, electric, of the nerve 213
spreading of, with decrement 238, 276
of the medium (pH) 13, 29
REDOX POTENTIAL 28
REFRACTIVITY 53, 67, 74, 83, 224, 245, 257, 259, 261
absolute, upon decremental conduction 239
of stimulation, in formation 357
and cathodic depression 360
REFRACTORY PHASE, absolute, upon spread of excitation 237, 276
relative, upon spread of excitation 237, 241, 276
RANVIER'S nodes 188, 190, 201, 217, 218
RESISTANCE of the protoplasm, increase in after excitation 101
RESORCIN, distribution between the simple coacervate and the liquid at equilibrium 169
REVERSIBILITY of signs of excitation of protoplasm 13
of protein denaturation, heterodromic 17
homodromic 17
RHEOBASE (Lapicque) 295
see Constant 'b'
change of, upon sectioning of the nerve 306
RUBIDIUM CHLORIDE, narcosis, and in effect on paranecrosis and stimulation of muscles 66
RULE of Arndt-Schultz 55, 69, 70
of constancy of the threshold energy of current 338
RHYTHM of automatic activity, dependence on lability 224
RHYTHMIC activity of fibers, duration of wave 269, 270, 271
of denervated muscles 273
duration of wave 265
of muscle fibers of the frog 272
of muscles, appearance on increase in excitability 224
following action of calcium-precipitating agents 224, 268
dehydrating agents 224, 268
on isolated nerve fiber of the crab 272
of a nerve, synchronization of excitation cycles
of the fibers 265, 276
of the rhythm of fibers, on electric excitation 267, 272, 276
appearance of, upon increase in excitability 262, 265, 271, 276, 370
during recovery from narcosis 262
of nerve endings of receptors 273
centers 274
of conducting fibers, relationship to increase in excitability 263
of the retractor of the worm Priapulus caudatus 272
of the heart, dependence on excitability 224
spontaneous, upon increase in excitability 262
SAFETY FACTOR 241
SERIES of Heber for cations and anions 180
SERIES of substances, according to the intensity of dehydration of muscles 115, 119
of killed muscles 119
of egg cells of marine animals 118
of nonelectrolytes, according to the intensity of dehydration by isomolar concentrations 115, 117
permeability (Overton) 105, 106, 114
of salts, by their action on the salt potential of the coacervate 183
of the muscle 180
SIGNIFICANCE, statistical, of the result 28
SHOCK, experimental (Cannon's method) 91
SHUNT 287, 334, 362
SODIUM AZIDE 79
bicarbonate 79
oxalate, effect on nerve excitability 266, 267
rhythmic activity of nerve, caused by 267
Salicylate, denaturation of hemoglobin by 17
sulfate, increase of muscle excitability by 273
distribution between a simple coacervate and the liquid at equilibrium 169
cyanide, effect of, on injury potential of muscles 205
citrate, rhythmic activity of nerve caused by 268
increase in muscle excitability 267, 273
SODIUM BROMIDE, narcosis, paranecrosis and stimulation of muscles 66
in muscles, three fractions 154
exit from muscles, rate of 146, 154
erythrocytes 144
diffusion of, in cuttle-fish fibers, rate of 150
hydroxide 79
effect of, on the mouse pancreas 97
distribution coefficient K for muscles 161, 175
of muscles, exchange with the medium, rate of 154
equation of 154
exchange with potassium in erythrocytes 144
penetration into cells 13, 144, 167, 189
into muscle fibers 146, 158
into muscles of warmblooded animals 146, 158
into erythrocytes 144
bond with the protoplasm of egg cells 163
with proteins in muscle fibers 162, 163
sulfate, narcosis, paranecrosis and stimulation of muscles 66
increase of excitability of muscles 273

content of in giant nerve fibers of squid 149
of muscle, in three fractions 154
fluoride, effect on muscles 206
chemical bond with the protoplasm 151
chloride, causing rhythmic activity of the nerve 268
effect on the mouse pancreas 98
on the negativity of the cell surface 189
threshold concentration of, for cell narcosis (muscles) 64, 65
SOLUBILITY of substances in dispersal state in protoplasm 127
SOLUTION, isomolar 118, 180
isotonic 120, 121
SORPTION of vital stains 16, 28, 29, 61, 64, 85, 141
acid 29, 141
basic 29, 138, 141
phase of decrease 44, 45, 47, 102
of stain by gland cells, increase in staining affinity with secretion 99
enhancement by killing the cells 140
SOUNDS, audible, effect on proteins, of 33
on muscle 33-36, 66
on protoplasm 16, 32, 34, 36, 39
as a cell irritant 12, 16, 32
mechanism of action of, on the protoplasm 39
SPERMATOZOA, suspension, vital staining of 30
metabolism of 80
STAIN, brilliant cresyl blue 20
in the cell, diffuse, upon excitation 32
indigo carmine 94
congo red 21, 23, 24, 26
crystal violet 21
malachite green 21
methylene blue 19, 20, 23, 28, 31, 79, 88, 93, 193
transition of, to the leucoform 28, 210
methyl violet 23
neutral red 23, 28, 29, 33, 35, 43, 46, 72, 83, 88, 89, 91-94, 96, 97, 99, 100, 139, 208
toxicity of 29
Nile blue 21, 23
rhodamine 139
toluidine blue 20
trypan blue 20, 73
phenol red 29, 38, 43, 46, 93, 139, 141, 142
cyanol 20, 23, 29, 33, 35, 88, 92, 94
Janus green 72
STAINING affinity of ganglia of mammals, biphasicity upon excitation by induction current through the nerve 92
of muscles, biphasicity upon denervation 102
under the influence of rise in temperature 67
of hydrochloric acid 66
upon narcotization by potassium chloride 65
sodium chloride 65
hydrochloric acid 66
differences in intensity of, in tetanus and in tonus 95
of nerve tissue, upon conditioned reflex
excitation 93
of protoplasm, biphasicity under the action of pressure 44, 45, 102
decrease in 31, 44, 45, 92, 101, 103
STAINING, vital, quantitative method of 28, 31, 33, 38, 42, 93
reversibility of 13, 141
of the mouse brain 92
of the mouse pancreas, intensification upon stimulation 88, 97
of tissue by acid dyes 29, 32
by basic dyes 29, 32
STAINS, vital 13
increase in binding of by the protoplasm upon excitation of the cell 13, 28-32, 89-96
diffusion of, into gelatin from a mixture with proteins 21
carbonyl, transformation of 22
acid 13, 20, 29, 32, 33, 38, 88, 94, 139, 142
distribution coefficient K of 141, 161
lack of granule formation of 32, 139
basic 13, 20, 28, 29, 33, 88, 139, 142
penetration into the cell of 30, 138, 141
distribution between muscles and medium 139, 141
solubility in lipids 138
series of, according to the binding capacity for proteins 22
diffusion rate of, into tissues 29
absorption spectrum of, in solutions 21, 24
of the triphenylmethane group 21, 22
three forms of existence in the protoplasm 142
extraction of, by acidified alcohol 28, 140
STIMULATION, subthreshold 214
STIMULUS, electrical (see Excitation)
"STRUCTURAL ENERGY" 211
SUBORDINATION, mechanism of 314
according to Lapicque 306, 312, 313
differences in coldblooded and warmblooded animals 313
SUCROSE, effect of, on the volume of muscles 115
isotonic solution and frog muscles 115
SUCROSE distribution between the complex coacervate and medium 169, 170
muscles and the medium 129
molecular weight of 119
adsorption limit A_∞ in muscles 174
SUGAR, penetration into the cell, dependence on concentration in the medium 133, 170
content of in erythrocytes, depending on concentration in the medium 129
SUGARS, nonfermentable, permeation into yeasts 133, 134
into muscles 125
into erythrocytes 123
permeation into muscles 124
into erythrocytes 123
SUSPENSIONS of tissues (cells) 30, 121
TAXIS, various types of 49

TEMPERATURE, effect on excitability, and dependence on conditions of excitation 314
of frog nerve 317, 318
method of measurement 315
of coldblooded animals 314, 325
of the tortoise 324
of frog heart 314
electroconductivity of nerve 318
increased, effect on the mouse pancreas 98
reversibility of paranecrosis 15
TETANUS 42, 53, 77
closing and opening, in the vicinity of a cross-section 263
as a series of contractions 77
of the muscle upon the action of sodium chloride on the nerve 263
of drying of the nerve 263
metabolism during 75, 77
THEORY of bioelectric potentials of Beutner 179
of Hermann 187
of Hertz 204
of Danielli 203
membrane 179, 181, 186, 197, 200, 215, 236
of Nachmanson 207, 209
oxidative-reductive 209
phase 179, 180, 186, 188, 211
of excitation, protein 237, 372
gradual 235, 238, 276, 277, 373
graphic illustration of 222
and damage, denaturation (Nasonov and Aleksandrov) 77, 90, 176
condensation (Chagovets, Hill, Ebbecke) 286
concentration of Nernst 286
membrane 104, 236, 372
of Segal 221
"selective pump mechanisms" or the "pump" theory 150, 176, 177
condensation (Hermann, Chagovets, Lapicque, Ebbecke, Hill and others) 335, 338, 339
concentration of Nernst 335, 339
of the lipoprotein complex of Lepeshkin 167
of "small currents", propagation of stimuli, of Hermann 216, 217, 221, 249, 369
of muscle contraction of Szent Gyorgyi 152
of the "sodium pump" of Hodgkin 151, 153, 173
of transmission of excitation 207
of potential of damage of Chagovets 185
of conduction, gradual 275
electric 219
of permeability of Hober 106
lipid 106
of Lundegaard and Crogg 108, 109, 173
membrane 104, 121, 135, 139, 143, 145, 148, 172, 173
mosaic 107
of Rosenberg and Wilbrand 109, 173
sorption 105, 121, 158, 173, 176, 177, 178
of excitation, denaturation 15, 18, 33, 39
of spread of excitation of Hermann (see theory of small currents)
gradual 216
resonance (sound) of Helmholtz 41
of the "sieve" 145
structure of protoplasm of Fisher and Zur 167
of phase potentials of Nernst 181
of the electric mechanism of spread of damage (Rothschue) 52
THERMONARCOSIS 67
THIOL POISONS 57
THRESHOLD of automatic activity 271, 274
value of, graphic representation (S-shaped curve) 223, 278
of excitability, protracted (see Constant 'b')
dependence on interelectrode distance 339-341, 343, 351
to induction shocks from DuBois-Reymond coil 263
short-term (see Constant 'a')
decrease in, after disconnecting cathode of polarizing current 358, 363
increase in, after closing circuit of cathode of polarizing current 358, 359, 361, 362, 363, 366
decrease in, after closing the circuit of the anode of polarizing current 365
of excitation, dependence on the amount of electricity 334
on voltage of stimulating current 334
on intensity of stimulating current 334, 344
of a single muscle fiber, dependence on energy of stimulating current 351
upon "intrusion" of current 359
dependence of value on changes in intensity of current 279
on the time of passage of current 279
measurement of, with a galvanometer in microamperes (Rushton) 334
of narcotization (of a narcotic effect) 57, 61, 64, 65
of damage 54
of spreading excitation 200, 276
THRESHOLD of spreading impulse as a criterion of excitability 277
of contraction of fibrocytes 73
THRESHOLD time of excitation (see Constant 'a')
amount of electricity (Q) 335
voltage, for human skin 278
dependence of, on external resistance of the circuit 343-345, 347, 351
on internal resistance of the nerve 347
on time of action of the stimulating current 278
on the interelectrode distance 342, 344, 345, 346
TIME of stimulation according to Lucas 295
TITRATION, electrometric 27
TISSUES, nonconducting, metabolism of 80
TONOPLAST of plant cells 110, 132
TONUS of constrictor muscles of shell of mollusks 53
TONUS-LIKE contraction, method of obtaining in a muscle (according to Zhukov) 78

TROPISMS, types of 49
TRYPSIN, heated denaturation, reversibility homodromic 17
UREA, effect of, on the volume of muscles 115
  protein denaturation by 17, 131
  distribution coefficient Q, of 130
  molecular weight of 119
  penetration into the cell, of 129
URETHANE, effect on the frog nerve 300
VACUOLE of plant cells, changes in volume of 110, 111
    accumulation of substances in 132
VERONAL, distribution in the blood of 125
VITAL stains, see Stains, vital
VISCOSITY of protoplasm, changes on excitation, of 12, 90
"VITEID", see Theory of the lipoprotein complex of Lepeshkin
WATER, excretion of, from protoplasm 119
  of coacervate systems 127, 168, 171
  bound, of the coacervate 168
    of the protoplasm 112, 113, 122, 168
YEAST CELLS, solubility constant K of lactose 134
    distribution of chlorine in 160

1442