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PROTOREACTION OF PROTOPLASM

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Abstract - My goal is to describe briefly the universal cellular reaction (UCR) to external actions and agents. This general reaction was the main subject of investigation by the scientific school of the outstanding Russian cytologist, Dmitrii Nasonov (1895-1957). The UCR consists of two phases of complex changes in cellular viscosity and turbidity, in the cell's ability to bind vital dyes, in the resting membrane potential, and in cellular resistance to harmful actions. Works from the Nasonov School have shown that these changes are based on structural-functional transformations of many cell proteins that react uniformly to actions of different physical and chemical nature. In general, these complex changes do not depend on cell type, indicating the universal and ancient nature of the UCR as well as its general biological significance. A new interpretation of the mechanism of the universal reaction is proposed in this paper, and a possible role for contractile proteins in the mechanism of the UCR of muscle cells is presented. In addition, the concept of cell hydrophobicity is introduced. Nasonov's School proposed a concept of physiological standardization that allows comparison of data obtained by different investigators and that will also be described here.

Key words: Cell hydrophobicity, contractile proteins, cytoskeleton, dye adsorption, general anesthetics, ionophore, limiting proteins, protoplasm viscosity, valinomycin, Overton-Meyer rule

"Sapere aude! Have courage to use your own understanding"!
Immanuel Kant

INTRODUCTION

According to an old Indian parable, well known in Russia, residents of the city of blind people asked several respected citizens to act as experts and to describe to them the nature of an elephant, about which they had heard much. It happened that one of these animals was present near the walls of their city. One expert who examined the elephant's leg by feeling it came to the conclusion that the elephant was a column. Another expert, upon touching carefully the animal's tail, stated that the elephant was a rope. The expert who got the tusk was absolutely sure that the elephant resembled a ploughshare. Clearly, the experts failed to agree and continued to dispute all their lives, since each one felt that their case was based firmly on established facts. Thus, each of them was in the right, but all of them were wrong on the whole.

Cell physiology and the scientists dealing with study of this discipline somewhat remind us of the meaning of this parable. To some of them, cell physiology focuses on the plasma membrane, to others the nucleus is the key, yet others prefer seeing the key to the mysteries to be found in signaling pathways. The "touching" of individual cell parts continues in contemporary cell biology.

Fortunately, the cell itself gives us examples of its reactions that imply the basis for generalizations, for a broad view of cell physiology. One such example is the universal cellular reaction (UCR) to external actions, which was studied in detail by the physiological school of the outstanding Russian scientist, Dmitrii Nasonov (1895-1957), founder of the Institute of Cytology of the Russian Academy of Sciences, and author of 117 publications including two monographs. At present, the total number of publications from the Nasonov School is estimated to be between 400 and 500. It is true that Nasonov himself called this reaction unspecific, rather than universal. But I consider the term "universal" to be more accurate and to better reflect the physiological and biological *significance* of this reaction, and I will apply this terminology here. The

Abbreviations: **AIH**: association-induction hypothesis; **UCR**: the universal cellular reaction

UCR is the uniform complex of substantial changes, apparently occurring in all cell types, in response to external actions of all kinds. The goal of this article is to describe some of these forgotten investigations, and to consider them in terms of another paradigm, the Association-Induction Hypothesis (AIH, 14,15) that seems to me to be a suitable basis for such an analysis. The necessity to reinterpret the results of the Nasonov's School and its heritage seems reasonable because the corresponding literature, already old, can be found to contain only the *phenomenological* or quite *general* accounts of the UCR. However, it seems to me that something better can be suggested in terms of contemporary biology. I hope the reader will agree that, in the framework of this brief paper, only a *schema* of this new approach to the problem can be presented. I will consider this task completed if I manage to present to the reader at least the general notion of the universal cellular reaction, and of its possible mechanism.

A UNIVERSAL REACTION OF THE LIVING CELL

One of the least understood properties of the living cell, apparently outside the scope of modern science, is its ability to respond to stimuli of *different* natures by *the same* standard complex of structural and functional responses. It is upon this phenomenon that the main efforts of Nasonov's School were focused. In these studies major attention was devoted to *changes* in cell properties, rather than to descriptions of its steady states. A simple but quite efficient method to investigate cell changes was to study binding of vital (non-toxic) dyes by cells. This procedure became the key approach in studies by the School and was also accompanied by studies of such physical characteristics as turbidity (transparency) of cytoplasm and nucleoplasm, their viscosity, biopotentials, and resistance to damaging actions by the agents discussed below.

The list of actions on the cells that were studied included: increased temperature, mechanical stress, hydrostatic pressure, electric current, general anesthetics, pH, medium tonicity, salts of heavy metals, hypoxia, and sound irradiation (200-7000 Hz, 94 dB). These studies used epithelial, nerve, muscle, connective tissue, the germ cells of various worms, echinoderms, coelenterates, molluscs, crustaceans, insects, and other invertebrates, as well as representatives of protozoa and some plant cells (see 20 for references).

Based on these abundant data, I present in Fig. 1 a universal complex of cellular changes in response to the agents named above. It includes changes of cell properties in the first phase and then in the second phase of both types of responses.

Changes of the first type

Many works have established that changes in turbidity of the cytoplasm and nucleus *always* occur in response to various actions on the cell. The second phase of the reaction is easily observed under the microscope: first the entire cell starts fluorescing with a pale blue light, then white structures appear, and turbidity increases. These changes are especially evident in nuclei, in which they appear even earlier than in cytoplasm. During the first phase of the reaction, the transparency of the protoplasm increases and, being a visual response, is best recorded by instrumental methods. In this paper the term "protoplasm" will be used, as it was in the days of Nasonov, to refer to the entire living substance of cells. On the whole, these changes can be characterized as follows: the size of intracellular colloids initially decreases, and later, at the second phase of the reaction, begins to increase, seemingly due to aggregation of the cytomatrix.

Another typical change characterizing the UCR response is an increase in intracellular viscosity. Not infrequently, it becomes possible to record a decrease of the viscosity (the first phase of type 1) before the beginning of its increase (the second phase).

Nasonov's School studied in the greatest detail the ability of cells to bind vital dyes. At rest, the cell *is almost never* stained with vital dyes, and this is especially true for the nucleus. However, under certain actions, the nucleus

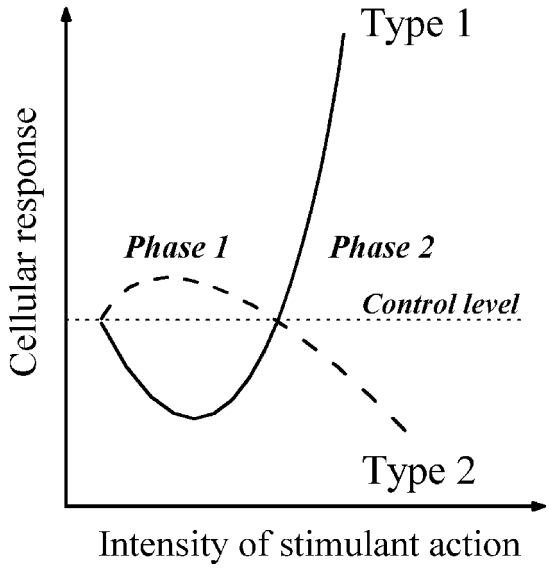

Fig. 1 Schematic presentation of the synchronous changes in cells that develop during the course of the universal cell reaction, in response to actions of various kinds. Changes in the cell's turbidity and viscosity, and of its ability to bind vital dyes, occur as described by type 1. Changes in cell resistance to harmful agents, and of the resting membrane potential, occur as in type 2. Further details are given in the text.

and cytoplasm start adsorbing the dye intensively, and then dye adsorption increases many times (up to 500% of the control or resting level, see Fig. 1). Especially intensively stained are the structures that are found in the nucleus, such as chromatin granules, nucleolus and nuclear envelope. In contrast it was found that during phase 1 the ability of cells to bind dyes decreased by 10-30%. In both cases, the % values refer to the *degree* of dye binding by all of the cells in the population studied.

Changes of the second type

Early in Nasonov's career, great interest was given to the data involved with the first phase of type 2 of the universal reaction – namely, the increase in resistance of cells damaged by heat or chemicals. This increase in resistance and stability was manifested, in particular, by an increase in the ability of isolated muscle to survive in Ringer's solution. Such stabilization of muscle and other cells was observed under the action of D_2O, general anesthetics and a variety of sugars, salts, vital dyes, and other compounds at concentrations at which development of the UCR was *delayed* at the first phase. At a higher doses (concentrations) the increase in resistance is replaced by its decrease during development of the second phase of the UCR. In that case, the cells become much more sensitive to damaging agents (see 28 for references).

Study by the School on the cellular resting membrane potential, recorded by extra- and intracellular methods showed that membrane hyperpolarization (relative to the resting state) took place during the early stages of development of the reaction. Later, after a longer or more intensive action (i.e. at the second phase) depolarization then begins (see 28 for references). Such results can be added to other characteristics of the UCR. For example, during the second phase an acidification of the nucleus and cytoplasm occurs, as well as the release from the cell of various substances including K^+ along with the simultaneous influx of Na^+ and Cl^- (20).

It should be noted that the first phase of the UCR is less intense and of shorter duration than the second phase, therefore, its recording requires a high precision experiment.

A matter of principle importance should be especially emphasized: *the universal reaction can develop not only in the cell as a whole, but also in its individual parts, depending on the nature of the action.* Hence, the UCR can also be a *localized process*. This peculiarity fascinated Nasonov and was always at the center of his attention; he believed that there was no principal difference between the localized reaction and the reaction of the whole cell in terms of the spreading excitation of the action potential (20).

Finally, after cessation of a given action on the cell, all subsequent changes show a reversed pattern and the cell gradually returns to the resting state. In particular, dyes are released by the cell into the surrounding solution against their concentration gradients during recovery. The cytoplasm and nucleus revert to being colorless, and K^+, various phosphates and other substances that left the cell are now taken up once more.

These changes can be summarized as follows: the first phase of the UCR is characterized by an increase in cell stability, an elevated resting membrane potential, and a decrease in cellular viscosity and turbidity, as well as a slight decrease in the ability of the cell to bind vital dyes.

The second phase is characterized by a decrease of cell stability and resting potential, a rise of viscosity and turbidity of the protoplasm, and a significant increase in the ability of the cytoplasm and nucleus to bind vital dyes.

WHY PROTOREACTION?

Experimental information accumulated over 40 years of investigations allowed Nasonov to conclude that his universal cellular reaction is based on reversible changes of cellular proteins (20). Indeed, changes in protein solutions *in vitro* are qualitatively similar to changes observed in living cells under comparable conditions. Thus, proteins lose solubility and aggregate, often with a rise in the viscosity of their solutions, and their ability to bind dyes increases when stressed. On the other hand, the actions that increase cell resistance also increase the stability of isolated proteins. Thus, agents such as ethanol and chloral hydrate at a concentration at which they increase resistance of the frog *sartorius* muscle also increase stability of the glycerinated *sartorius* muscle models (29), as well as of isolated actomyosin (16,17). Those are important and possibly profound observations.

The above changes in protein solutions are as universal as the UCR and they are induced by the actions of practically any physical or chemical agent. The opposite is also true: thus, agents able to produce these changes in proteins *in vitro* also elicit the UCR (20). Comparing these many observations, the conclusion was easily reached that even the very first proteinoids (6) in evolution had the capability to produce the universal reaction, and that has general biological significance (20). It is in this context that I have referred to the universal reaction as the "*protoreaction*", as it is this response that must be the basis through evolution for the formation of numerous regulatory systems in cells and, to a degree, will continue to be reflected in physiological reactions in contemporary cells. But this term also has another meaning: in the protoreaction, we should find the fundamental processes that must be responsible for the physical basis of life. So what is this physical basis?

PHYSIOLOGICAL ATOM OF THE LIVING CELL

Experts who consider that cell physiology is very heavily influenced by membrane biology will hardly set about explaining the mechanism of protoreaction, since we have already stated that it takes place not only in whole cells, but also in local intracellular areas as well as in membrane-deprived structures such as glycerinated cell models and isolated proteins. For this reason, a promising basis for analysis of protoreaction is, in my opinion, Ling's AIH that has been developed by its author for 4 decades and strives to be revolutionary, a break-through in viewpoints on the cell based on its bulk-phase system (15,19).

According to Ling's theory, the physical basis for life is an ion-water-protein complex – the smallest structural unit that has the capability for protoreaction:

$$K^+\text{-}H_2O\text{-}PROTEIN_{unf}\text{-}ATP \rightleftharpoons PROTEIN_f + H_2O + ADP + P_i + K^+,$$

where $PROTEIN_{unf}$ represents unfolded protein molecules, whose polypeptide chains are accessible to the solvent water; where K^+-, H_2O-, ATP– represent protein-bound potassium ions, water, and ATP; and $PROTEIN_f$ the folded protein molecule, in which a significant part of the polypeptide chain becomes inaccessible to water (see Fig. 44 in reference 15 for further details).

The left part of this equation refers to a cell in the *resting state*, and the right part to the state of activity or excitation. According to the AIH, it is such local changes that occur during action potentials, muscle contractions, and other forms of cellular activity. Transitions from the resting to the active are accompanied by the release of free energy necessary to perform biological work (15).

Transitions between these two states of the ion-water-protein complex represent, basically, a sol-gel transition or a cooperative phase transition. According to this view, the triggering switches between these phases are what generates the dynamics of life. These transitions are based on regulated conformational protein changes that are not simply related to shifts of atoms. It is probably more useful to evaluate relative conformational changes by their accompanying thermodynamic changes rather than by values of mechanistic shifts of parts of the molecules. If that approach is taken, the ion-water-protein complexes and their protoreaction are in essence the physiological "atoms" of the cell in the sense that this is the *minimal* structural entity able to produce the main interactions responsible for cellular life, and its response to external disturbance. I suggest that the living cell acts as if it is composed of such "atoms", the various combinations of which are then included into organelles, the cytomatrix and various other cell structures. These "atoms" can acquire features of specialization, but the main structural-functional principles of their activity remain unchanged, so I will consider these "atoms" to be the basic units of the living cell. Finally, I should note that not only the whole protein molecule can act as a basic unit, but also that parts of it can operate that way. In addition, when associations of such "atoms" take place with a high degree of cooperativity, these "associations" or "complexes" can be regarded, in some cases, as one "atom".

This is the AIH logic, as I understand it. It seems to me that, on the whole, shifts of dynamic equilibrium between two states of the basic unit reproduce, at the *elementary level*, the protoreaction of cells, as shown in Fig. 1. I suggest that these dynamics are the two states of a binary code, upon which cell physiology operates.

Among other things I will later use the studies on dye adsorption done by the Nasonov School to illustrate the basic features of the protoreaction. But first it is necessary to examine a question not usually considered in this fashion: what is the nature of cell hydrophobicity?

CELL HYDROPHOBICITY: A MISSED ROLE FOR PROTEINS

For a long time, and up to the present, the term hydrophobicity was mostly has been associated chiefly with lipids. The well-known Meyer-Overton rule was always a strong argument in favor of the lipid nature of biomembranes and of the membrane theory of anesthesia. Until the 1960s, to be "hydrophobic" was synonymous with being "lipid", and the hydrophobic properties of the cell were explained by the presence of its lipid membranes, first of all, and primarily the plasma membrane. Indeed, based on these concepts, numerous "lipid" theories of anesthesia were put forward.

However, in the 1960s, when studying thermodynamic characteristics of the thermodynamics of protein folding and unfolding, Brandts (3) was the first to prove convincingly that during the folding of a protein molecule, hydrophobic areas are formed internally which are inaccessible to water. Initially the thermodynamics of conformational transitions in proteins was the subject of study by a small group of specialists. However, with time, it has become evident that hydrophobic areas within cells are represented not only by lipids, as this was thought for more than 70 years, but also by proteins. The importance of this reappraisal is emphasized by the fact that, after water, protein is the most abundant of all other constituents, comprising up to 65% of the dry mass of cells, and greatly exceeds the total amount of lipid. What I propose here is that the volume of the hydrophobic *protein* phase can greatly exceed that of the hydrophobic lipid phase. However, I also recognize that the full significance of this observation has not been understood and seemingly not accepted by contemporary cell physiologists in terms of

paradigms and working hypotheses.

The next development essential in our understanding of cell hydrophobicity came from the works of Katz and Simon (11) and Halsey *et al*. (9), who came to the principally important conclusion that there was *no difference* between the physical properties of hydrophobic sites of lipids and proteins as revealed by a thorough thermodynamic analysis. In other words, hydrophobic compounds within cells will interact with *any* other hydrophobic site, regardless of location be it in proteins or in lipids. This statement has an important consequence that will become clear when we consider the example of valinomycin, a selective potassium ionophore. It is accepted as *axiomatic* that this rather hydrophobic compound is dissolved only in the lipid phase of the cell's plasma membrane, and becomes a K^+ carrier by virtue of its concentration gradient. As a result and as repeatedly observed, cells treated with valinomycin loses K^+. This "dogma" first appeared over 50 years ago when nothing was known about the hydrophobic phase(s) in proteins, and still persists to this day (10,25). But we also know now that such overly simplistic interpretations of valinomycin's effect on the cells are quite unacceptable. At present, it is evident that valinomycin can be inserted into *any* hydrophobic phase**,** regardless of its nature, be that lipid or protein. Hence, valinomycin can essentially change properties not only of membranes, but also of proteins (including those of the cytomatrix**)**; therefore, it is no longer correct to explain the mechanism of action of this compound on the cell**s** *only* by the action on changes in the permeability of the plasma membrane. Interestingly, this statement, made on the basis of general considerations, has become now been confirmed experimentally. It turned out initially that valinomycin also had peculiar "side effects". Thus, it was revealed that valinomycin had the ability to interact directly with cytochrome c oxidase (21,26,27), Ca^{2+}-ATPase (2), and (Ca^{2+},Mg^{2+})-ATPase of skeletal muscle sarcoplasmic reticulum (5). It seems reasonable to suppose that other even partially hydrophobic ionophores might also directly interact with proteins. That topic seems worthy of further careful study.

Thus, after decades, it seems that the Meyer-Overton rule is neither a proof of the lipid nature of membranes, nor evidence for the key role of membrane lipids in anesthesiology. This rule merely indicates a role for hydrophobic interactions in the cell permeability to the so-called lipophilic compounds.

The term "hydrophobic interaction" often is considered to be synonymous with non-specificity. In reality, that term of hydrophobic interactions is as non-informative about the degree of their specificity as is the use of such terms as hydrogen bonds or ionic interactions. All these terms merely indicate the physical nature of the interaction, rather than indicate any degree of the level of their specificity. The latter quality depends on numerous additional factors that are realized in the microenvironments of the interacting molecules.

At present, the protein theory of anesthesia is commonly accepted, according to which the targets of the anesthetic effect are hydrophobic sites located in proteins (7), and this is of principal importance for the issues I consider in this paper.

PHASE TRANSITIONS OF BASIC UNITS AND CELL HYDROPHOBICITY

The evidence at my disposal suggests that the basic unit protoreaction, apart from other changes, leads to the appearance in the cell of a *new physico-chemical factor* – hydrophobic areas formed by proteins. This statement is based on postulated properties of the basic units, according to which a shift of the dynamic equilibrium between two states of the basic unit (unfolded $\rightleftharpoons$ folded) to the right will bring about a relative increase in the number of protein molecules in the folded state. This will favor the formation of protein hydrophobic sites (areas, domains, pockets) by virtue of the participation of hydrophobic side groups, both inside the protein molecule and in intermolecular contacts (3). Thus, Ling's model predicts that at transition of the protoreaction into the second phase of its development (see Fig. 1), the volume of the cellular protein hydrophobic phase will increase. However, it is to be stressed that Ling does not consider such a possibility in his extensive writings (14,15).

An increase of the hydrophobic phase volume fundamentally changes the conditions of the intracellular environment and inevitably leads to a massive redistribution of *all* lipophilic compounds within the cell and between the cell and the external medium. Such a redistribution should also involve key substances such as ATP, since this compound is distinguished by significant hydrophobicity (13). That seems to be a rather significant point with regard to the UCR.

During Nasonov's time, information on the properties of proteins was scarce. It was cautiously believed by his School that development of the protoreaction leads to the appearance of additional fixed charges on proteins in cells, with which vital dyes, known to be organic ions, presumably interacted. However, in the review by Leo *et al.* (13) it is pointed out that all vital dyes are characterized by high lipophilicity, whereas the *charge* on these compounds produces no essential effect on their hydrophobic interactions with other substances. This result is particularly true for organic *cations* (24). One of these organic cations, the vital dye neutral red, was widely studied by the Nasonov School, and its use allowed them to obtain most of the data on an increase of dye binding by the cell during the second phase of the protoreaction. Of

great importance in this connection, is the fact that neutral red is no different from general anesthetics (17) as far as its mechanism of interaction with cell structures is concerned: both the dye and general anesthetics interact with cell hydrophobic sites. Thus, vital dyes are, in essence, *indicators of the volume of the cell hydrophobic phase formed by intracellular proteins*.

Nasonov explained the increase in dye binding in the course of the protoreaction as being due to the "initial stage of protein denaturation", since proteins denaturated *in vitro* also bind dyes better than their native conformations. Both in Nasonov's works and in the context of the present paper, use the term "denaturation" (i.e. loss of natural properties) seems inappropriate, as it implies irreversible and probably lethal changes. In discussions between Nasonov and his opponents, it was argued that the cell is able to repair "denatured" proteins, and specifically those with conformational modifications *similar* to the denaturated state. However, from the point of view of the above-considered dynamics of the basic unit states, restoration of the cell to its initial state after protoreaction looks not so much like *reparation*, but more like the normal change of the basic unit states involved in mechanism of UCR. Inappropriateness of the term "denaturation" was also indicated by numerous data obtained by Nasonov and his colleagues, according to which the *normal* functional activity of cells (secretion, muscle contraction, nerve impulse propagation, transmission of synaptosome signals, etc.) is also accompanied by an increase in the cellular viscosity, turbidity and dye binding (see 20 for references). Of great interest is the question of how vital dyes *leave* cells, against their concentration gradients, after completion of the protoreaction and a return of the cells to their resting state. First, it could be because a transition to the resting state is accompanied by a decrease in the volume of the hydrophobic phase (i.e. a decrease in the number of the dye-binding hydrophobic centers). Second, according to the AIH, a large fraction of cell water in the resting condition is in a state of restricted mobility ("bound") and is a poor solvent for large ions and various molecules (15). As a result, these are excluded from intracellular water into the surrounding solution. On the other hand, if we interpret the data according to the membrane theory, it becomes necessary to postulate the existence of active transport systems for each of the dyes studied by Nasonov's School.

The concept of the basic unit helps explain as well the first phase of protoreaction when the ability of a cell to adsorb dyes is slightly reduced. The general explanation is based on the assumption that the cell contains a small number of basic units in a folded state under resting conditions since the balance "unfolded units $\rightleftharpoons$ folded units" is dynamic. If some influence on a cell leads to an even greater displacement of the dynamic balance to the left, the total volume of protein hydrophobic phases in a cell will decrease *in comparison* with the resting state. As a result, the cell's ability to bind lipophilic dyes will also decrease. For example, in the case of the action of general anesthetics interacting hydrophobically with cellular proteins, thermodynamic factors could play an important role. For example, at a certain anesthetic concentration it could be advantageous thermodynamically for protein hydrophobic side groups to make contact with the mixed solvent (water + anesthetic) instead of with each other (3). As a result, folded conformations of basic units, available in the resting state of a cell, could become unstable and unfold, and expose its hydrophobic groups to the mixed solvent. In that fashion, the dynamic balance between the two states of the basic unit will be shifted to the left to a greater degree than in the resting condition.

Another important factor in these processes is an increase of cellular ATP during the first phase of the protoreaction (see 28 for references). According to the AIH, an increase in cellular ATP concentration should lead to a shift to the left of the equilibrium between the two states of the basic unit. Ling (15) believes that ATP is the "cardinal adsorbent" and a key component of the AIH. In the context of my paper an increase in ATP concentration would strongly affect the dynamic equilibrium between the two states of the basic unit: an increase in ATP concentration would shift the equilibrium to the left, while a decrease would shift the equilibrium to the right.

The significance of the increase in hydrophobicity of the cytoplasm and nucleus for the functions of the cytoskeleton, signaling pathways, genome, and other important cellular mechanisms remains virtually unknown and has yet to be investigated.

INTRACELLULAR VISCOSITY

I should first note that the studies done by the Nasonov School involved descriptions of macroviscosity due to limitations in the methodology of his era. Changes in the cytoskeleton are the first that come to mind as an explanation for the changes in viscosity during the course of the protoreaction. However, years of study on the effects of anesthetics on cytoskeletal elements have shown that these compounds depolymerize microtubules and microfilaments at clinical concentrations (1). Thus, at the phase of cellular narcosis (i.e. at the second phase of the protoreaction) when the viscosity increases, this is opposite to what would be expected from disassembly of the cytoskeleton.

Taking into account the basic unit properties, another explanation could involve the bound state of intracellular K^+. During tetanic contraction of muscle and ethanol exposure, under conditions when the muscle cell protoreaction reaches the second phase of its development,

K^+ is known to leave the muscle due to K^+ desorption from the K^+-binding matrix (30). K^+ efflux from muscle during excitation is a well-known. In the AIH context, free anionic groups on proteins produced by K^+ desorption interact with fixed cationic groups on the same protein, or adjacent ones. As a result of these interactions of fixed ions, there appears a three-dimensional network of protein molecules bound to each other in the cell, or in localized parts of it. This network is believed to increase the viscosity significantly. A role in the stabilization of such a network can also be played by interprotein hydrophobic interactions, where hydrophobic side groups of adjacent protein molecules interact with each other, thereby contributing to the stabilization of protein complexes or aggregates. Taking into account the high protein concentration in cells, this "polymerization" of basic units can proceed very fast, and involve large parts of cells or even their entire volume. Such aggregations will inevitably lead to an elevation of viscosity, an increase in the sizes of intracellular particles, and, hence, to an increase of cell turbidity. Taking all this into account, the cytoskeleton does not seem to play the key role in mechanisms underlying the increase in viscosity.

Recall that, during the first phase of protoreaction, the viscosity and turbidity fall below their resting levels. One can account for those observations by a process involving the absorption. To do so, extra anionic groups fixed to the basic unit are needed, some of which come from sites that were previously occupied by other fixed charges during the resting state. According to AIH logic, the number of fixed anionic groups available for K^+ binding increases when the cellular ATP concentration rises. This theoretical prediction is in accord with the above-mentioned data showing an increase in ATP during the first phase of the protoreaction (see 28 for references). Thus, an increase in ATP synthesis and its excessive binding (compared with the resting state) by basic units results in the breakage of an additional number of ionic bonds *between* proteins, and an increase in the number of fixed anionic groups that can bind K^+. It is further proposed that the above is accompanied by a partial "depolymerization" of the three-dimensional network of protein molecules, because some of the ionic bonds participating in its stabilization are broken. Such a process of weakening of interprotein interactions would also be reflected as a decrease in cell viscosity and an increase in its transparency as a result of the dissociation of protein aggregates.

Unfortunately, cell viscosity and K^+ content, as far as I know, have always been studied separately. Consequently, one can only refer to indirect evidence in favor of the above-described mechanism. Such indirect evidence comes from an interesting work by Troshina (31) showing that, under the action of insulin on frog *sartorius* muscle, the resting potential of the muscle fibers increases, while their ability to adsorb neutral red decreases; hence, insulin produces the first phase of the protoreaction in this muscle, during which viscosity and turbidity of the sarcoplasm are known to decrease. On the other hand, it is well established that insulin increases the K^+ content in muscle (4) which, according to the AIH, could be due to the appearance of additional sites for K^+ binding, and to a corresponding decrease in stability of the protein matrix, as discussed above. As a result, the dynamic equilibrium in the basic unit shifts to the left to a greater degree than in the resting state, leading to a decrease in viscosity and the ability to bind vital dyes.

It seems that the same effect can be produced by any action that increases cellular ATP content since this increase is accompanied by a rise in intracellular K^+ content (8). In this connection, it is interesting that these actions (classical for Nasonov's School) lead to an increase in creatine phosphate and ATP in the cell, since these also increase cell resistance / stability (see 28 for references). Based on the above discussion, the following "rule" can be formulated: the greater the shift of dynamic equilibrium between two states of basic units toward the left, the higher the cell resistance and stability.

Thus, *major cause of changes in colloidal properties of cells, including rheological ones, seems to be assigned to the state of K^+-binding by the cellular matrix, the extent of which differs at different phases of the protoreaction*.

LIMITING PROTEINS

From the point of view of the AIH, basic units play the key role in maintaining fundamental physico-chemical conditions of the intracellular medium, which underlie the entire structural-functional organization of cells. This gives good grounds to the belief that the loss by basic units of the ability to perform their function would be sufficient for cell death. If so, the proteins that are the structural basis of these units can be called "limiting proteins" – those that play a critically important role in providing the necessary conditions for metabolism and, therefore, life.

This theoretical anticipation has been confirmed experimentally. Rosenberg *et al*. (23) studied the kinetic parameters of thermal protein denaturation and thermal death of unicellular and multicellular organisms. They came to the paradoxical conclusion that denaturation of one protein, or of a small number of proteins with close properties (that the authors called *limiting proteins*) were sufficient for thermal death of a cell or organism. From the point of view of the AIH, such proteins might be those of the K^+-binding cell matrix. An important question arises: what can be said about the nature of these proteins?

As already noted here, the first protoreaction phase is characterized by an increase in cell resistance to damaging factors, including thermal damage. For instance, in

Ringer's solution containing 6 mM chloral hydrate or 680 mM ethanol the survival time of frog *sartorius* muscle is twice as long as that of control preparations. Similar effects have also been obtained using other chemical agents (see 28 for references). The question then is: which intracellular structures and/or proteins are the targets of the actions responsible for an increase in resistance of the muscle cell? Of course, there are many proteins in cells, and their properties differ greatly. For instance, the maximal stabilizing effect of ethanol on ribonuclease is achieved at 2000 mM ethanol (3), whereas 680 mM is sufficient in the case of actomyosin (16).

In this connection, it is interesting to compare data obtained on *living muscle* and *glycerol-treated muscle models* (see 28 for references), and on isolated *actomyosin* (12,16,17). It has been established that stability of all these preparations increased over *the same concentration range* for chloral hydrate (maximum effect at 6 mM) and ethanol (maximum effect at 680 mM). In other words, this response of the living cell is, to some degree, *reproduced* by isolated proteins, specifically, by the contractile muscle proteins. This astonishing observation merits more detailed study.

But why does actomyosin give such a good correlation with living muscle in terms of these effects? Is this because of the high content of these proteins in muscle? Or do the contractile proteins play some additional key role in enabling viability of muscle cells? One possible answer might be connected to the fact that the contractile proteins bind the majority of K^+ present in muscle (see 15 for references) and thereby are the structural origins of the basic units of the UCR in muscle cells. If this is really so, then the contractile proteins represent the K^+-binding matrix, whose stability is entirely responsible for cell viability. In that case, it is clear that inactivation of the K^+-binding matrix alone could make functioning of muscle cells impossible. And, to the contrary, actions that stabilize contractile proteins *in vivo* also make the treated muscle cell more resistant to malfunction. Apart from the key role of contractile proteins as the basic units, they also play an important role in the transmission of signals within muscle cells (18). Under such circumstances, and in this context, contractile proteins can indeed be considered limiting, and the above-mentioned experimental data provide additional evidence in support of the conclusions of Rosenberg *et al*. (23) about the cause of the thermal death of cells and multicellular organisms.

PROTOREACTION AS A PHYSIOLOGICAL STANDARD

It is easy to see that the protoreaction represents a non-linear response of cells to some action. This means that the same stimulus can produce different results depending on its intensity. This partly explains numerous controversies in the literature, as authors studying some particular property of the cell do not suspect that under their experimental conditions, the protoreaction can develop, so that the cell properties being studied depend essentially on the phase involved. Analysis of the results obtained, without considering the physiological background under which they are obtained, is not likely to be correct. So it is important to know in which state of protoreaction cells are when they are being studied. Indeed, it is very likely that protoreaction takes place in every case if a cell is affected by any method. One can only compare those effects that are developed against a background of the *same phase* of protoreaction (see Fig. 1) according to the rule "all other conditions should be equal" (ceteris paribus). In this way, numerous cell effects could be standardized, depending on the protoreaction phase in which they were observed.

In my opinion, the best indicator of the protoreaction is a change in the hydrophobicity of cells or of certain intracellular structures. Thus, an increase in nuclear hydrophobicity might initiate some reactions, while preventing others. For example, it is very unlikely that signalling systems in cells will operate similarly in the hydrophilic (phase 1) and hydrophobic (phase 2) regions of cytoplasm or nucleus. All these issues are extremely interesting and important to increase of effectiveness of science, but are almost entirely uninvestigated.

Why can the protoreaction be used as a standard? Because the entire body of scientific evidence accumulated by Nasonov's School supports the claim, with some degree of certainty, that the protoreaction is *the only cell reaction that, in spite of its complexity, has a universal and general biological character. Furthermore, the complex changes occurring during development of the protoreaction appear in all cell types, at the scale of the entire cell as well as intracellular structures, including molecular complexes.* The structural-functional principles that underlie the protoreaction can be revealed in greatly different ways in the nucleus, cytoplasm, organelles, during muscle contraction, nerve impulse propagation, apoptosis, and so on, but the principles themselves remain invariant.

CONCLUSION

Currently, the ideas, approaches and methods of study developed by Nasonov's School have essentially been forgotten. But it is absolutely clear to those who still remember this page of history of Russian science that the School studied some *fundamental* cell properties, whose significance for biology is not understood up to the present time. It is necessary to continue these investigations of the Nasonov School, at the least because Nature never disappoints those who study successively its fundamental manifestations. In my view, one such manifestation is undoubtedly the protoreaction.

Here I have outlined merely the general scheme of the UCR / protoreaction and its possible interpretation based on the AIH of Gilbert Ling. It is certainly evident that many aspects of this approach need further study and experimental confirmation. But something else also seems evident: only after carefully comparing the findings of the Nasonov School with the main features of the AIH, which I tried to do here, does it becomes clear as to which issues need further study. Formation of a plan of investigation is one of the challenges of a good theory.

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