Plant Physiology

A TREATISE

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CONTENTS

PREAMBLE TO CHAPTER 1

From the first observations of protoplasm and of cells, special attention has been paid to their boundary surfaces and to the controlled entry and exit of substances which is an essential feature of the cell in life and the loss of which is an accompaniment of injury and death. Therefore, having treated cells, in Volume I, as organized units and having surveyed their ability to negotiate energy transformations through their metabolism, their ability to control the passage of substances across boundary surfaces, or membranes, needs now to be discussed as a very fundamental characteristic of cells. This is done in Chapter 1. While general principles of cell permeability are being sought, the great variety of cells and environments which are encountered in the plant kingdom needs to be recognized. Furthermore, it should also be recognized that cells which are in their most physiologically active, or growing, state possess properties additional to those of quiescent cells. Nevertheless, there is a vast body of information which relates to those passive permeability properties of cells that may influence the entry of solutes into, and their egress from, cells. These questions will now be discussed with special reference to the organization and properties of the boundary surfaces in cells, where these properties are believed to reside.

CHAPTER ONE

Cell Membranes: Their Resistance to Penetration and Their Capacity for Transport

RUNAR COLLANDER

I. Introduction

Every living cell exists in a unique situation. On the one hand, its interior must be kept well isolated from its surroundings. This is imperative for many reasons. Imagine, for instance, a unicellular freshwater organism. If the surface layers were freely permeable to dissolved substances, then solutes could not be retained in the cell. No turgor pressure could be maintained in such a cell. Moreover, the sensitive vital machinery, with its multitude of enzymes requiring more or less constant hydrogen ion concentrations for their proper functioning, would be exposed to all the vagaries of the milieu. The orderly, integrated, and specific course of the life processes, so characteristic of all living organisms, would, under such conditions, be rendered impossible. Almost the same holds true of the cells of a multicellular plant: in order to be able to work successfully they must be effectively differentiated from their surroundings, that is, from the aqueous solution which bathes the cell walls.

On the other hand, every living cell must maintain a continuous exchange of substances with its surroundings: it needs oxygen for respiration; it must take up diverse nutrients; and it must get rid of the waste products of metabolism. Furthermore, in a multicellular plant numerous substances must be transported, say, from the photosynthesizing cells to the cells of the storage tissues, and from these to the meristematic cells and to others which consume organic foodstuffs.

Nature thus imposes two conflicting demands upon all living cells. From this point of view their life may be regarded as a perpetual cruise between Scylla and Charybdis. But how do they resolve this intricate situation? On the following pages we shall try to throw some light on this question.[1]

[1] For further particulars, and a more detailed bibliography, the reader is referred to the following comprehensive monographs: Stiles (157), Brooks and Brooks (25), Davson and Danielli (47), and W. Ruhland, ed., "Handbuch der Pflanzenphysiologie —Encyclopedia of Plant Physiology," Vol. 2. Springer, Berlin, 1956.

Living cells or, more properly speaking, the living protoplasts, are in fact isolated from their surroundings in such a way that the diffusion of numerous substances, outward and inward, is almost entirely prevented. At the same time the protoplasts are freely permeable to numerous other substances. Yet other substances behave in an intermediate manner in this respect. But we shall also find that the protoplasts have mechanisms at their disposal that make possible the active transport of certain substances, without regard to the direction in which these substances would tend to diffuse spontaneously.

This whole problem is often spoken of as the "permeability problem." This is not, however, a very appropriate designation, for it does not place the stress on the proper point. By far the most remarkable features are the very effective isolation of the protoplasts and also their striking capacity for active transport of substances even against concentration gradients.

II. The "Permeability Problem": A Historical Sketch

One of the very first to draw attention to the effective isolation of plant protoplasts was the Swiss botanist Carl Nägeli (114). As early as 1855, and thus only a few years after the recognition of protoplasm as the true site of the life processes, he published his observations on the impermeability of the protoplasm to the red, purple, or blue anthocyanins which occur dissolved in the cell sap of so many plants. In the same publication Nägeli described plasmolysis, a phenomenon that was to be important in later studies of the permeability properties of plant protoplasts. Nägeli's interpretation of this process was, on the whole, correct, for he realized that the plasmolytic contraction of the protoplast is due to an osmotic withdrawal of water from the cell sap. Only on a minor point was he mistaken, for he shared an idea then current that every osmotic process is composed of two opposing flows, endosmosis and exosmosis, which, depending on the qualities of the membrane and also on the composition of the solutions, bear a certain quantitative relation to each other.

Twelve years later Wilhelm Hofmeister (88) stressed that in many cases only an exchange of water between the protoplast and the surrounding medium is possible. He thus realized that, toward many solutions, protoplasts behave like semipermeable membranes. (The actual word "semipermeable," however, was only coined several years later by van't Hoff.)

Hugo de Vries found in 1871 that the protoplasts of the beet (*Beta vulgaris*) root, when plasmolyzed in a sodium chloride solution, for instance, will maintain their volume unaltered even for weeks. From this

he concluded that these protoplasts must be considered practically impermeable to sodium chloride, for, if the protoplasts were even faintly permeable to the salt, during the long period of this experiment it would penetrate the protoplasts to an appreciable extent and thus raise their osmotic value. But this would have resulted in an increase of their volume, for exosmosis of sugar, which is the principal solute in the cell sap, could not be detected.

Even somewhat earlier, in the middle of the 1860's, Moritz Traube (162), a German-Jewish tradesman who in his spare time made several far-reaching scientific discoveries, had produced the first artificial semipermeable membranes and made preliminary studies on their properties. Wilhelm Pfeffer made a more thorough investigation of one of these, the copper ferrocyanide membrane. His studies, together with an abundance of new fundamental facts and ideas, were published in his book "Osmotische Unteruchungen," which appeared in 1877. Among other things, he pointed out that the high resistance of the protoplasts to diffusing substances is principally due to the extremely thin plasma membranes (*Plasmahaut*), the one surrounding the whole protoplast, the other situated at the interface between the protoplasm and the cell sap. In the same book (130), Pfeffer offered a general theory of the penetration of water and solutes through membranes of limited, or selective, permeability. He stressed that permeation does not depend only, as Traube had assumed, on the respective dimensions of the membrane pores and of the permeating molecules. Another important factor, according to Pfeffer, is the affinity of the diffusing substance for the membrane, i.e., its ability to dissolve in, or react reversibly with the membrane material. Moreover, if the membrane is provided with water-filled pores, the diffusion through these pores will also be influenced by the interfacial forces acting at the water-membrane interface. A permeability theory worked out eighty years ago was bound to be somewhat speculative; nevertheless we must admire the comprehensiveness of this early theory. It was, in fact, so general that most, if not all, of the permeability theories proposed later may be regarded, as special cases of the all-embracing theory of Pfeffer.

As early as the 1870's and 1880's observations showed that water is not the only substance which penetrates the living protoplasts readily. Thus de Vries, in his paper of 1871, showed that the protoplasts of the common beet, in a quite intact state, are rapidly penetrated by ammonia, as shown by the color change of the cells when they are immersed in very dilute ammoniacal solutions. In 1886, Pfeffer (131) observed that several basic dyes are also taken up rapidly by intact plant protoplasts and even accumulate in very high concentrations in

the sap of many cells. He also found that retarding the vital activity of the cells, by low temperatures or by narcotics, had little effect upon the uptake of these dyes. Pfeffer therefore concluded, and rightly so, that the uptake of these dyes does not involve the intervention of an active transport mechanism. In the following year, Klebs (104) observed that if *Zygnema* sp. cells are put into a 10% glycerol solution, they will first undergo plasmolysis and then gradually recover. Obviously then glycerol penetrates the protoplasts with considerable rapidity, although by no means as rapidly as water. The analogous behavior of the epidermal cells of *Rhoeo discolor* toward glycerol and urea solutions was reported by de Vries in 1888 (170).

In spite of such scattered observations on the degree of permeability of plant protoplasts toward various solutes, it is scarcely an exaggeration to state that it was with the investigations of E. Overton from about 1890 onward that the permeability problem entered a new phase. Overton was the first to study cell permeability systematically and the first to state, explicitly, the nature of cell permeability and to distinguish this from the permeability properties of inanimate membranes.

Charles Ernest Overton, a distant relation of Charles Darwin, had been educated in Switzerland where, in the nineties he had a position as *Privatdozent* of biology at the University of Zurich. Preoccupied with investigations concerning the mechanism of heredity, he intended to study the influence of ethyl alcohol on the sex cells. First he sought to ascertain whether this substance can enter these cells. The literature contained no data on this point, and so Overton was compelled to decide the question experimentally and found that the protoplasts are about as permeable to alcohol as to water. This observation led Overton to study the permeability of the protoplasts toward other substances. Having discovered that the permeability of protoplasm to different solutes varies between very wide limits, Overton was gradually led to investigate what was the cause of this selective, or differential, permeability. With great tenacity Overton devoted the rest of his life to the elucidation of this problem and performed experiments with a very great variety of both animal and plant cells, testing as many different substances as possible. By 1899 he had made a great many experiments with more than 500 different substances.

Overton was obviously deeply impressed by the great uniformity with regard to permeability shown by cells which are very different in other respects; e.g., muscles and erythrocytes, on the one hand, root hairs and algal filaments, on the other. He found that the introduction of certain radicals, such as the hydroxyl, the carboxyl, or the amino group into a permeating molecule always greatly decreased its perme-

ation power. Conversely, a lengthening of the carbon chain or an esterification of its carboxyl or hydroxyl groups enhanced the penetration power of a molecule. But how were these experimental results to be explained? In a paper read before the Naturforschende Gesellschaft in Zurich in 1898 and published the next year, Overton pointed out that there is a striking parallelism between the permeating power of different substances and their solubility in fats and fatlike solvents. However, it is not the absolute solubility in fats that is the decisive factor, but the relative solubility, i.e., the distribution, or partition, coefficient in the system fat-water. The smaller this coefficient, the more difficult is the passage of the substance through the protoplast. To explain these quite unexpected observations, Overton adopted Pfeffer's view that the diffusion resistance of the protoplast is, for the most part, located in the extremely thin plasma membranes. Overton now postulated that these membranes were impregnated with fatlike substances, or lipids.*

This was the fundamental theme of Overton's much discussed lipoidal theory of cell permeability, which immediately made the question of permeability a central problem in cell physiology. Numerous botanists, zoologists, medical men, pharmacologists, and chemists were attracted to the problem, but the progress in this field was not commensurate with the interest it aroused. On the contrary: during the first decades after the appearance of the lipoidal theory the discussion around it was characterized by a degree of confusion and passion rarely met in other fields of science. Many things contributed to this state of controversy.

In the first place, Overton unfortunately never published his experimental results in full, nor did he reply to the attacks on his theory. He was an extremely careful experimenter, but his magnum opus, intended to give a complete picture of the lipoidal theory and its experimental basis, was never completed, and only fragments of it were published (121–125). This made it difficult for other investigators to appreciate fully the firm foundation on which his views were based.

Furthermore, many of Overton's opponents failed to observe a fundamental distinction already made by Pfeffer and stressed again by Overton. From the very first Overton had emphasized that the interchange of substances between the cell and its surroundings comprises processes of two quite different kinds. According to Overton, this inter-

* Fats, the higher fatty acids, phosphatides, and sterols were formerly lumped together under the general name of lipoids, and this designation is often used even today, especially in the European literature. This group of substances is characterized by insolubility, or sparing solubility, in water and great solubility in "fat solvents," e.g., ether or chloroform. In accordance with the newer custom we shall here designate these substances as lipids.

change is partly regulated by the more or less passive resistance to diffusion of substances through the protoplasm. However, another part of the relations between the cell and its environment consists of an active transport of substances even in the reverse direction to that in which, from a purely physical standpoint, they would be expected to move. Thus fresh-water algae, for instance, take up salts from a very dilute solution, their natural living medium, and accumulate them in their cell sap. Such transport against the concentration gradient constitutes a certain amount of work done at the expense of energy released by the metabolic processes in the cell. Obviously the mechanism of such an active transport process must be fundamentally different from the mechanism of simple diffusion processes. In order to underline this difference, Overton coined a special term for this active transport, designating it as the adenoid (i.e., glandlike) activity of the cells. He stressed that the lipoidal theory aims solely at the elucidation of the passive permeability of the protoplasts, while their adenoid activity represents quite another problem.

Nevertheless, as already mentioned, many of Overton's opponents failed to discern, or at least to take into account, this fundamental distinction. Thus some of them pointed out that lipid-insoluble substances (e.g., sugars, amino acids, mineral salts) must also be taken up by the cells, and they thought that this fact alone would be enough to overthrow the lipoidal theory. Other writers looked upon the concept of adenoid activity as the manifestation of an obscure, unsound vitalism, and some even suspected that the adenoid activity doctrine had been invented as a mere screen to hide the shortcomings of the lipoidal theory. Today, however, there can no longer be any doubt concerning the general occurrence and great importance of active transport processes in plant cells. In fact, the concept of active transport constitutes an indispensable complement, not only to the lipoidal theory, but to every conceivable permeability theory in the proper sense of this word.

At all events, the lipoidal theory of cell permeability had to face a long-contested resistance before its validity, even in main outline, was generally recognized. Meanwhile several other permeability theories were energetically advocated. It will suffice, however, to mention here only three of these hypotheses.

(1) I. Traube, in a long series of papers, enthusiastically defended his "*Haftdruck*" or "retention pressure" theory, according to which the permeation of solutes depends on how great is their *Haftdruck* to the membrane substance as compared with that to water. Unfortunately, however, Traube was never able to make a clear statement of the true meaning of the word *Haftdruck*. Today, therefore, this theory is almost

forgotten. However, if we try to interpret it as benevolently as possible, we may perhaps take *Haftdruck* as a rough equivalent of "affinity" or "intermolecular attraction." Interpreted in this way, the theory of Traube is not very far from some modern views concerning the permeation mechanism.

(2) In order to account for the permeation of water, mineral salts, and other more or less lipid-insoluble substances, Nathansohn (115) postulated a plasma membrane composed of a mosaic of both lipids and proteins. On this view, lipid-soluble substances would enter the cell through the lipoidal parts of the membrane, while the lipid-insoluble substances would take the other route. This hypothesis explains why lipid-soluble substances are always able to penetrate the plasma membrane, while the uptake of others may vary considerably from time to time according to hypothetical changes in the protein part of the membrane. The objection may be raised to Nathansohn's theory that it is very speculative in nature. Besides, we now know that the uptake of mineral salts, for instance, is principally due to active transport, not to permeability in the strict sense of the word. Nevertheless the mosaic hypothesis, when accorded the best interpretation, may also perhaps be found to be not so very far from the truth.

(3) The ultrafiltration theory of W. Ruhland aroused still more discussion. In its original version (143, 144) it maintained that the permeation power of colloids depends on their state of dispersion: the greater their dispersion, the more readily they would, according to Ruhland, permeate into living cells. It will be noted that the ultrafiltration theory, in this form, said nothing about the principal point of the permeability problem, namely, how it comes about that living protoplasts are more or less impermeable to numerous noncolloidal substances. Moreover, if the permeation of highly dispersed colloids was due to their state of high dispersion, then it would be natural to conclude that all noncolloidal solutes, owing to their still greater dispersion, would have even more permeation power. Of course, such a conclusion was too absurd even in those days of confused thought in the field of cell permeability. It only shows that the ultrafiltration theory could scarcely be taken seriously before, in 1925, it appeared in an essentially new version.

The "new" ultrafiltration theory (146) was based on experiments carried out with the great, *Oscillatoria*-like "sulfur bacterium," *Beggiatoa mirabilis*. According to Ruhland and Hoffmann, its permeability toward nonelectrolytes and alkaloid salts was almost entirely dependent on the molecular volume of the permeating substance. Moreover, Ruhland and Hoffmann suggested that although their experiments had been

made with a single organism, and indeed with a rather unusual one, the ultrafiltration theory would, nevertheless, prove applicable in its essentials to all living protoplasts.

The first impression was, of course, that the ultrafiltration theory differs so radically from the lipoidal theory that they would be mutually exclusive. But, as we shall see, this is not so: for it seems now that molecular size cannot be entirely neglected in a comprehensive understanding of the permeation processes.

From about 1925 it has been increasingly clear that qualitative observations on permeability are not enough, but that quantitative measurements, as exact as possible, are needed. Numerous such investigations have, in fact, been carried out during the last thirty years, and as a consequence the confusion that had characterized the field of cell permeability has begun to dissipate, enabling established facts and assumptions to be distinguished, although by no means all differences of opinion have been eliminated.

In what follows we will attempt, primarily, to find out which points concerning cell permeability have so far been established with reasonable certainty, and after this has been done, we will try to draw deductions from these facts. First of all, however, let us take a look at the active transport processes accomplished by plant protoplasts.

III. Active Transport Processes[2]

The occurrence of specific active transport processes brought about by the energy released in cell metabolism was recognized by such pioneers of cytophysiology as Pfeffer (132) and Overton (121). However, most of their successors had little appreciation of the fundamental significance of these phenomena. It is true that Höber, in his "Physikalische Chemie der Zelle und der Gewebe" (73), which appeared in numerous editions from 1902 onward, always gave considerable weight to the idea of active transport, but the very term "physiological permeability" used by him was not likely to make clear the radical difference between active transport and simple permeation. And so it happened that when, some thirty years ago, Hoagland, Steward, Lundegårdh, and others again stressed the importance of active transport, their views had, to a large extent, the charm of novelty. It is only during the last ten or fifteen years, however, that the central role of active transport has been universally recognized, so that this phenomenon has at last received the attention it deserves.

[2] For further information concerning active transport, the reader is referred to Volume VIII of the "Symposia of the Society for Experimental Biology" (159), which is in its entirety devoted to this problem.

A. PERMEABILITY AND ACTIVE TRANSPORT: TERMINOLOGY

In the preceding historical sketch, it was pointed out that many disputes concerning the "permeability" of living cells have arisen because this word has been used in quite different ways. Even today misunderstandings in this field may arise from a variable, ill-defined terminology.

Used in its widest sense the term "permeability" embraces the total interchange of substances between the cell and its surroundings. Permeation, taken in this sense, includes both active transport processes and diffusion. On the other hand, when used with their restricted meaning, the words permeation and permeability refer solely to those transfer processes in which the protoplast plays the passive role of a mere resistance to be overcome by the substance as it leaves or enters the cell. Permeability in this latter sense thus equals $1/R$, where R denotes the diffusion resistance of the cell.

Now, which terminology is to be preferred? Historically, the answer to this question is clear, for all the classical authors in this field, with Pfeffer and Overton leading the way, have used the words permeation, permeability, etc., in their restricted sense, and fortunately, it is more practical to retain this custom. For, if we should now decide to use these terms in a wider sense, we would be obliged to invent a new word for permeability in the narrower sense. On the other hand, permeability in its widest sense is synonymous with other terms such as transfer, exchange, uptake, absorption, flux, etc. Therefore, the present writer restricts the term permeability to its original meaning and speaks of the permeability of protoplasm in just the same sense as physicists and engineers speak of the permeability of inanimate membranes.

For active transport, several other names have also been used. One of the oldest is the term "adenoid activity" (121). In recent years the terms dynamic transfer, active secretion, metabolic, or nonosmotic transport have all come into prominence. Whereas nonosmotic transport should properly relate to the movement of water, it is largely a matter of taste which other term relates to the movement of solutes.

B. THE EXPERIMENTAL DISTINCTION BETWEEN PERMEATION AND ACTIVE TRANSPORT

Permeation processes always occur with the activity gradient or, in the case of ions, with the gradient of electrochemical potential. Hence in most cases permeation, like diffusion in general, tends to smooth out and finally to abolish any existing concentration differences.

On the other hand, neither the spontaneous, random thermal movements of the molecules nor the migration of ions under the influence of

electrical potential gradients suffice for the active transport processes. These processes imply the intervention of mechanisms by which energy set free in cell metabolism is made available for transport, which may even create great concentration differences between the cell sap and the external medium.

It is not always easy, however, to decide whether a particular transfer process is to be regarded as metabolic or passive. The mere fact that some substance seems to be accumulated in the sap does not necessarily imply that the process is "metabolic" in the strict sense. Thus basic dyes and other weak bases are often apparently accumulated, even very efficiently, in cell saps, and yet the process may be regarded as passive, rather than active, because the entering solute is bound in the cell [see Bogen (15) who uses the term metaosmotic in these cases]. Moreover, the well-known Donnan equilibriums, also occurring in inanimate systems, result in an uneven distribution of diffusible ions on the two sides of a membrane, but nevertheless they involve no direct metabolic activity.

The following criteria may be used to decide whether a given process is to be considered as passive or active, i.e., as a case of "permeation" or "active transport."

(1) The rate of a permeation process is, in most cases, directly proportional to the concentration gradient, but in the case of active transport this is not so.

(2) Chemically similar substances often mutually depress the active uptake of one another, presumably because they compete for one and the same transport mechanism. In the case of simple permeation such competitions scarcely occur.

(3) As we shall see, the permeation power of different substances is correlated with their lipid solubility and molecular size. Now, if it is found that the penetration rates of two substances of about the same solubility and molecular size (e.g., two optical isomers) differ markedly, this is a strong indication that the uptake of at least one of them is not due to permeation alone.

(4) Elimination of free oxygen should, in aerobic organisms, depress or even prevent active transport. Permeability, on the other hand, seems scarcely to be affected by the absence of oxygen, except in so far as the membrane may be structurally affected thereby.

(5) Active transport may be reversibly reduced by narcosis, while the effects of narcotics on permeation processes are more complex.

(6) Substances known to inhibit certain enzymes, such as hydrocyanic acid, carbon monoxide, sodium azide, dinitrophenol, iodoacetate, and fluoride, have been successfully used to show that particular en-

zymes are involved, directly or indirectly, in certain absorption processes. A pronounced effect of enzyme inhibitors on permeation processes, although conceivable, is not very probable.

Thus there are numerous more or less suitable criteria which discriminate between passive permeability and active transport. While no criterion suffices alone, if used in combination the criteria will, in most cases, be conclusive.

There are, however, certain rather difficult cases. One of the most puzzling is that described as "facilitated diffusion" by Danielli (44). Facilitated diffusion occurs, as with simple diffusion, under the driving force of thermal agitation, and the equilibrium reached is the same as that achieved by simple permeation. However, facilitated diffusion occurs over a limited fraction of the cell surface (at the so-called active patches) only and at any one site is, like an enzymatic process, restricted by both structural and steric factors, so that only particular molecular species are concerned. The occurrence of facilitated diffusion in plant protoplasts is, however, still hypothetical.

C. Active Transport of Different Substances

Active transport is most obvious in the case of strong electrolytes, i.e., in the case of ions. Active transport of ions is of such fundamental importance to the plant and is, at the same time, such a complicated phenomenon that it is treated in a special chapter of this treatise (see Chapter 4). In the present chapter we will concern ourselves mainly with the transport of essentially nonionized substances and of amino acids which, although strongly ionized, bear both positive and negative electric charges and are thus effectively electrically neutral (zwitter ions). Similarly, since activated movements of water are considered in Chapter 2 of this volume these will not be considered here.

1. Sugars

For a long time it has been surmised that sugars penetrate cells so slowly that they must resort to specific means to meet their requirements for these important nutrients.

Yeast cells afford an especially attractive object for studies on this problem, since they seem to be specialized for rapid uptake of sugar. It has been shown by Conway and Downey (40) and Rothstein (141), among others, that yeast cells are more or less impermeable to lactose, galactose, sorbose, and arabinose, while they will readily take up glucose, mannose, and fructose. Their ability to take up certain sugars but to reject others of similar lipid solubility and molecular size strongly suggests the existence of a special transport system for the sugars that

are absorbed. The mechanism has been investigated especially by Rothstein and his co-workers (141). The starting point was the observation that uranyl ions (UO_2^{++}) even in low concentrations strongly inhibit glucose fermentation. Moreover, it could be shown that the uranyl ions do not enter the interior of the yeast cells but are bound to specific sites at the cell surface. Furthermore, uranyl was found to be an inhibitor of reactions specific to the uptake of hexoses, while it is without effect on all the metabolic reactions involved in the respiration and fermentation of other substrates. The action of uranyl must thus "be confined specifically to those reactions occurring at the cell surface which introduce sugars into the metabolic machine, without any effect on the integrity of the machine itself, which is presumably located inside the cell" (141). Probably it acts by chelating with adenosine triphosphate (or other phosphorylating agent), and thus prevents the phosphorylation of the sugar which normally proceeds at the cell surface in connection with active sugar uptake.

In the cells of higher plants, also an uptake of sugars is often readily observed when, for instance, pieces of leaf are floated on sugar solutions (175). The slowing down of the process by anaerobic conditions indicates that it occurs predominantly by active transport, although a slow sugar uptake by diffusion may occur simultaneously. A still more striking example of active sugar transfer is the copious secretion of sugars in nectaries. Less striking, but physiologically highly important, is the accumulation of sucrose in the sieve tubes of photosynthesizing leaves, an indispensable first step in the removal of assimilates from the leaves (176). Owing to technical difficulties, the mechanism of active sugar transfer has not yet been as thoroughly analyzed in higher plants as in yeasts, but a prevalent idea is that it depends on phosphorylation and dephosphorylation processes, while Gauch and Dugger (63) have also suggested that the sugar transport may depend on the formation of a boric acid-containing complex.

2. Amino Acids

Arisz and his collaborators (2) have studied the uptake and transport of amino acids and amino acid amides by two plant organs, namely the leaf parenchyma of *Vallisneria spiralis* and the leaf tentacles of *Drosera capensis*. In both cases absorption was found to be of the "active" type, as shown by its dependence on the oxygen supply and its inhibition by several enzyme inhibitors.

Another important series of investigations into the uptake of amino acids has been carried out by Gale and co-workers (62). They found that if cells of *Staphylococcus aureus* which are free of glutamic acid

are suspended in a solution of glutamic acid, there is no accumulation of this substance within the cells as long as precautions are taken to exclude metabolic sources of energy. If, however, an energy-yielding substrate such as glucose is added, then rapid accumulation of glutamic acid takes place within the cells and the internal concentration may rise to as much as 400 times that of the external medium. The accumulation is abolished by any inhibitor which prevents the metabolism of glucose. Moreover, the rate of accumulation is independent of external concentration except for very low values of the latter. Finally, if cells rich in glutamic acid are washed and resuspended in water, there is only a very slow loss of glutamic acid to the external medium. There is thus a strong indication that the glutamic acid accumulation taking place in these cells is the result of active transport (cf. 112a).

3. *Other Substances*

For some twenty years it has been known that diatoms, when plasmolyzed in solutions of sugars, polyhydric alcohols, amides, etc., will deplasmolyze with much greater rapidity than other plant cells in the same situation. This has so far been interpreted as an indication of an exceptionally high permeability of the diatom protoplasts. However, according to Bogen and Follmann (19), the recovery from plasmolysis is largely prevented by enzyme inhibitors. This suggests that the normal deplasmolysis of the diatoms is to a corresponding extent due to active uptake of the solutes tested. A similar, although less striking, influence of metabolic inhibitors has been reported by the school of Bogen in other kinds of cells also. Bogen (16) therefore concludes that active transport is of much wider occurrence than has hitherto been assumed and that it occurs with a great variety of substances.

It seems that these claims will necessitate a critical re-examination of the question of the relative importance of active transport, on the one hand, and simple permeation, on the other. Until this has been done, the present writer is inclined to hold to the classical view that only slowly permeating substances are to any considerable extent subject to active transport. At any rate, it is obvious that the greater the permeability to a given substance, the greater also is the risk that this substance will leak out from the cell by diffusion and so nullify the results of the active accumulation process (cf. 85b).

IV. Diffusion in Inanimate Systems

It is not impossible that the penetration of water through some protoplasts is, to a certain extent, a filtration process, that is, a bulk flow of water through submicroscopic pores (cf. 128, 166). Mostly, however,

permeation through plasma membranes is due predominantly or even solely, to diffusion.

To understand the diffusion of substances across plasma membranes one should examine what is known about diffusion in general and especially diffusion through artificial membranes which are better known as to structure and chemical composition than the plasma membranes.

A. DIFFUSION IN HOMOGENEOUS MEDIA

Diffusion is due to the spontaneous thermal movement of molecules and ions. Random movement of the particles leads ultimately to an even distribution within a single phase.

In 1855, Fick proposed the equation.

$$dS = -Da\frac{dv}{dx}dt$$

where dS is the quantity of substance which in the time dt passes across an area a in which dc/dx is the concentration gradient (i.e., the change in concentration with distance). D is a constant of proportionality and is called the diffusion coefficient. It gives the amount of substance diffusing across unit cross section in unit time when the concentration gradient is unity. The negative sign appears because diffusion takes place from a region of higher to one of lower concentration. The diffusion coefficient has the dimensions: area divided by time; it may thus be expressed, e.g., as square centimeters per second. A very exhaustive exposition of the application of Fick's law to cell physiological problems is that of Jacobs (96).

The Fick equation applies just as well to liquid and solid systems as to gaseous ones. In other respects, however, there are considerable differences between diffusion processes in these systems. Thus, in gaseous systems the molecules are subject to only small intermolecular forces and are therefore always free to move. In liquids and solids, on the other hand, there are considerable forces holding the molecules together in a more or less rigid framework. In such systems, therefore, a molecule can only diffuse if it has sufficient kinetic energy to overcome the forces holding it to its neighbors and if in addition it has sufficient energy to push other molecules out of the way. Each molecule will thus mostly vibrate about a mean position and only occasionally jump to a new one. The energy necessary for such a jump is called the "activation energy of the diffusion." Its magnitude will be the greater the stronger the bonds between the diffusing molecule and those of the medium and

also the stronger the attraction of the molecules of the medium for each other, that is, the greater the viscosity of the medium. Thus, if the attraction is due to comparatively weak van der Waals' forces the diffusion will, other things being equal, be more rapid than when strong hydrogen bonds are involved. According to Danielli (42), the magnitude of $DM^{1/3}$ (where D is the diffusion coefficient and M the molecular weight of the diffusing substance) will be approximately constant for diffusion processes in aqueous solutions and in media of still lower viscosity, but this will not be the case in more viscous liquids nor in solids. On the other hand, the diffusion in gels is by no means as slow as one might be inclined to assume from the stiffness of the gel. This is due to the fact that the gel is traversed in all directions by innumerable water-filled channels in which most substances are able to diffuse almost as in ordinary water. Only when the diameter of the diffusing particles approaches the diameter of the channels is the diffusion rate considerably decreased.

B. Diffusion through Membranes

Reference here may be made to the excellent monographs of Höber (75), Dean (48), and Pappenheimer (128).

In attempting to explain how a substance can penetrate a membrane by diffusion, we may have recourse to two quite different principles. If the membrane consists of a homogeneous layer of a water-immiscible substance, its permeability toward different substances is principally dependent on the extent to which these substances are soluble in the membrane substance. If, however, the membrane has a sievelike structure, similar to a filter, diffusion will take place, either exclusively or principally, across the channels which join the aqueous phases on the two sides of the membrane. Cases intermediate between these two also occur.

1. Homogeneous, Solventlike Membranes

If we have a membrane as a separate phase between two aqueous solutions, we may picture the permeation of a substance through this homogeneous membrane as composed of three successive steps: the permeator (the penetrating substance) has first to dissolve in the membrane substance on one side of the membrane, then to diffuse through the membrane to the other side, and, finally, to pass from the membrane phase into the second aqueous phase. The first process—the distribution of the permeator between the aqueous phase and the membrane phase—is obviously dependent on the magnitude of its dis-

tribution coefficient, k, defined by the equation

$$k = \frac{\text{conc. in membrane phase}}{\text{conc. in water}}$$

The rate of the second process—the diffusion across the membrane—is primarily dependent on the absolute difference in concentration of the permeator inside the two boundaries of the membrane. Substances having a distribution coefficient smaller than unity will therefore diffuse across the membrane in accordance with their distribution coefficient.*

Under many conditions the diffusion rate is thus almost directly proportional to the distribution coefficient of the permeator. Admittedly, the diffusion rate is also dependent on the viscosity of the membrane substance and on the molecular size of the permeator. However, even with great differences in molecular size, diffusion rates do not differ very much, the diffusion coefficients being, as we have seen, more or less inversely proportional to the square root of the molecular weight. On the other hand, the distribution coefficients of different substances often differ enormously. Their magnitude may therefore mostly be considered as the deciding factor.

A homogeneous membrane may, however, also be regarded as a "potential energy barrier" (42). This means that before a permeating molecule can enter the membrane phase from an aqueous solution it must acquire sufficient kinetic energy both to overcome the forces holding it to the water molecules and also to make a gap in the nonaqueous layer. Now, the more hydrophilic the permeator is—that is, the more firmly its molecules are attached to the water molecules—the greater, of course, is the kinetic energy required to bring about detachment from the aqueous phase. And since a large amount of kinetic energy is seldom available, the process of transition will be the slower the more hydrophilic is the permeator. When the permeating molecule has crossed the nonaqueous layer, it finally has to diffuse across the oil-water interface into the water. For hydrophilic molecules, however, this is easy.

It will be noted that the concept of the membrane as a potential energy barrier is by no means in conflict with the picture of the permeation process previously given. They are two different, but

* At this point for the sake of clarity, a warning to the reader is perhaps required. Although it was said above that substances having a low distribution coefficient will diffuse comparatively slowly across the membrane, this does not imply that the movements of the single permeating molecules are retarded but only that the number of these molecules in the membrane is reduced and that, in consequence of this, the quantity of the substance passing across the membrane in unit time is decreased. Perhaps it would therefore be better to speak of a decrease in the flux instead of a decrease in the diffusion rate.

substances are decidedly surface-active and rather hydrophobic, while formamide and urea are surface-inactive and strongly hydrophilic. It is therefore probable that permeation through the narrow-pored collodion membrane is also influenced by adsorption and/or solubility phenomena which will favor the penetration of surface-active, hydrophobic solutes.

The classical copper ferrocyanide membrane is probably a more typical molecular sieve, for its permeability toward surface-active, hydrophobic substances seems not to be greater than toward surface-inactive ones.

Very interesting observations have been made on the penetration of nonelectrolytes into crystalline zeolites after the water normally present in the interstices within the crystals had been removed (9). It was found that zeolites possessing a fairly open kind of framework take up *n*-paraffins but not isoparaffins. Those of a slightly higher density do not take up either *n*- nor isoparaffins, except methane and ethane. Finally, the crystals with the narrowest interstices are only penetrated to a negligible extent by methane and ethane but take up molecular nitrogen and oxygen very rapidly. From these observations it can be concluded that the *shape* rather than the *volume* of the penetrating molecules exercises an influence upon the penetration power. The feature deciding the uptake or nonuptake is, above all, the cross section of the molecule, which is the same for all the *n*-paraffins, while the isoparaffin molecules have a somewhat greater cross section. Increasing the chain length does not materially influence the ultimate sorption equilibrium, but slows down the rate of intracrystalline diffusion. Such clear-cut results would scarcely have been possible if the interstices of a single zeolite crystal were not of very uniform diameter.

The permeability of sievelike membranes to ions has been extensively studied, especially by measuring the electrical potentials produced. Collodion membranes have proved especially useful in such studies (152). Slightly oxidized collodion (nitrocellulose) membranes contain negative charges arising from the ionization of scattered carboxyl groups situated in the pore walls. These fixed negative charges are combined with mobile ions of the opposite charge, say, sodium ions. Such a membrane will therefore be found permeable to sodium, and also to other, cations. At the same time it may be more or less impermeable to all anions, owing to the electrostatic repulsion exercised by the charges on the pore walls. The narrower the pores and the greater the density of the negative charges, of course, the more pronounced is this anion impermeability. The impermeability to anions makes the membranes impermeable to salts, too. However, the permeability of such a selec-

tively cation-permeable membrane toward different cations varies considerably, depending on the dimensions of the ions in question. Thus, for instance, Michaelis (cf. 75) found the relative mobilities of some cations to be as shown.

	H	Rb	K	Na	Li
(a) In free solution	4.9	1.04	1.00	0.65	0.52
(b) Within a dried collodion membrane	42.5	2.8	1.00	0.14	0.048

These results are readily understandable when we remember that the hydration of the alkali cations decreases in the order Li > Na > K > Rb and that the volume of the hydrated ions decreases in just the same order.

By impregnating collodion membranes with basic substances (alkaloids, basic dyes, basic proteins) they may be rendered electropositive. Such membranes are permeable to anions, but more or less impermeable to cations.

The selective ion permeability of the copper ferrocyanide membrane also depends upon the surface charge of the membrane and upon the charge, valency, and size of the diffusing ions (41). Thus the order of permeation power of some anions was found to be this: $Cl^- > Br^- > NO_3^- > I^- > IO_3^- > SO_4^{--} >$ oxalate$^{--} >$ ferrocyanide^{----}

A detailed theory of the ion permeability of electrically charged membranes has been worked out by K. H. Meyer and Teorell (cf. 160).

3. *Intermediate Cases*

As already stated, even a "homogeneous" membrane, though operating principally as a selective solvent, will at the same time show just a slight trace of sieve action in so far as larger molecules will, other things being equal penetrate the membrane somewhat more slowly than smaller ones. On the other hand, sievelike membranes will seldom select the diffusing molecules solely according to their size: in most cases the result will also be more or less influenced by some kind of "affinity" between the permeating molecules and the membrane substance.

There are, however, other membranes which are still more clearly intermediate between the two main types so far discussed. Thus, by adding lipids to a solution of nitrocellulose in alcohol- ether, membranes may easily be prepared which combine the selective solvent properties of the lipid with the sieve action of the pure collodion membrane (174).

The artificial membranes so far mentioned have all been of a considerable thickness. The plasma membranes, on the other hand, are probably lipid-containing films only a few molecules thick. From the standpoint of the study of cell permeability, therefore, it appears to be of the greatest interest to gain some insight into the permeability properties of lipid films of the latter order of thickness. It is very regrettable that technical difficulties have so far largely prevented the study of the permeability properties of such membranes. As an example of the surprises possible in this field, it may be mentioned that it was only quite recently that the first reliable measurements of the resistance offered by fatty acid monolayers to the evaporation of water were carried out (1). According to this investigation such layers decrease evaporation by a factor of about 10^4, while in earlier measurements of this sort almost no resistance at all to the water evaporation had been found.

A film, a few molecules thick and consisting of arachinic acid and cadmium arachinate, has been found almost impermeable to ions but fairly permeable to H_2S and I_2 molecules (11). From the standpoint of cell physiology it is of the utmost importance that such investigations should be performed, quantitatively, with different types of films a few molecules thick and with a greater variety of permeators. To this end the films should, if possible, be spread between two aqueous solutions, though this would be extremely difficult.

V. Measurement and Quantitative Expression of Permeability and Active Transport

A. TERMINOLOGY

The terms permeation and permeability are often used synonymously, but this is not admissible. Permeation refers to a *process*, while permeability is a *property* of a membrane (cf. digestion—digestibility, etc.). This distinction is even clearer when we realize that the permeation rate always depends on two quite different factors, namely, (a) the permeability of the membrane and (b) the driving force of the process. Only too often the importance of the factor (b) has been underestimated or even neglected in relation to the factor (a). And yet it should be clear that each of them is essential to any actual permeation process.

Moreover, it is not desirable to speak of the permeability of a solute. The ability of a substance to penetrate a membrane is more properly called its permeation power.

B. PROTOPLASMIC PERMEABILITY[3]

Imagine that a protoplast is put into a solution of a substance whose concentration at the surface of the protoplast is kept constant by continual mixing. Let us further assume that no active transport occurs and that the substance permeates relatively slowly through a thin surface layer of the protoplast, whilst the diffusion resistance in the interior of the protoplast is negligible. Obviously, then, the diffusion resistance of the surface layer alone controls the total permeation rate. By applying Fick's law we arrive at the equation

$$\frac{dS}{dt} = P \times A(C - c) \qquad (1)$$

where dS is the amount of substance entering the cell in time dt; A is the area of the cell surface; C, the equilibrium concentration of the permeator; and c, its concentration at any given time. P, the *permeability constant*, is a measure of the permeability of the protoplasmic surface layer. It may also be said to be a measure of the permeation power of the permeator in question. From this point of view it may be more logical to call it the *permeation constant*. Generally the equilibrium concentration (C) of the permeant in the cell sap is approximately equal to its constant concentration in the external solution. As far as our present knowledge goes, the exceptions to this rule are more apparent than real. Thus, in the case of basic dyes and other weak bases, which are often accumulated very effectively in the cell sap, it must be remembered that while the substance enters the cell as a free base, it is mostly not accumulated as such but is bound to an acidic constituent of the sap.

If V is the volume of the protoplast, $c = S/V$. If we assume that V and A remain constant during the permeation process, equation (*1*) can be integrated and becomes:

$$P = \frac{V}{At} \ln \frac{C}{C - c} \qquad (2)$$

The permeability constant, or permeation constant, P, has the dimensions length divided by time or, in the natural absolute CGS system, centimeters by seconds. The diffusion coefficient, on the other hand, as already stated, has the dimensions $l^2 \times t^{-1}$. The difference is

[3] For a more detailed mathematical treatment of the permeation processes, including methods of calculating permeability constants, see Rashevsky and Landahl (137), Jacobs (99), and especially the exhaustive treatise of Stadelmann (154).

due to the fact that the thickness of the diffusion-resisting surface layer is generally not known and must therefore be incorporated in the constant P.

In deriving equation (*2*) we have assumed, for the sake of simplicity, that the permeation rate is controlled solely by a thin surface of the protoplast. Now, strictly speaking, this assumption never holds true. First, because in most cases there is not one plasma membrane only, but two: one outer and one inner one. What is actually aimed at in the great majority of permeability experiments is thus to measure the sum of their diffusion resistances. Moreover, there is an unstirred layer of cell sap inside the protoplast and there is in most cases a cell wall outside it; it is self-evident that these must contribute more or less to the total resistance which the permeator has to overcome. The thicker and more impervious the cell wall, the bulkier the cell sap vacuole; and the smaller the diffusion resistance of the protoplast, the more, of course, will the experimentally found value of P differ from the true permeability of the protoplast.

There is, however, one means of correcting the experimentally measured P values to some extent, namely this: The total diffusion resistance of the cell is approximately equal to the sum of several successive resistance, viz., those of the cell wall, the plasma membranes, the mesoplasm, and the cell sap. Now, when the cell is killed, the diffusion resistance of the plasma membranes toward many substances is greatly reduced, while the other diffusion resistances will remain almost unaltered. The difference between the resistance of the cell in the living and dead state can therefore be used as an approximate measure of the resistance of the undamaged protoplasmic membranes:

$$R_{\text{plasma membranes}} \approx R_{\text{living cell}} - R_{\text{dead cell}}$$

or

$$\frac{1}{P_{\text{plasma membranes}}} \approx \frac{1}{P_{\text{living cell}}} - \frac{1}{P_{\text{dead cell}}}$$

Disregarding experimental errors, the permeability constants expressed in, say centimeters per second, are true measures of the protoplasmic permeability and thus can be directly compared.

For certain purposes, however, other measures of permeability may profitably be employed. Thus, the half-time of the permeation process, i.e., the time in which the initial concentration difference between the outer solution and the cell sap is reduced by half, may be found to serve as a measure of the rapidity of the permeation process. It should, however, be borne in mind that the half-times depend not only on the magnitude of the cell permeability but also on the dimensions of the

cells. Thus, a *Micrococcus* cell only 1 μ in diameter will display half-saturation times 10,000 times smaller than a *Valonia* cell measuring 1 cm in diameter, provided the permeability constants are the same in the two cases. As a matter of fact, the great rapidity with which many interchanges occur between most plant cells and their environment is determined, to a greater extent than is often realized, by the very great relative surface of the majority of cells.

Finally, the water permeability may be expressed by a special water permeability constant indicating the amount of water that will pass across unit cell surface area in unit time per atmosphere of pressure difference. Such water permeability constants are not, of course, comparable with the permeation constants of solutes.

In the case of active transport processes there is hardly any other measure of their intensity than the flux, i.e., the amount of substance taken up in unit time per unit area of the cell surface.

C. Experimental Methods of Measuring Permeability and Active Transport

There exist a very great number of methods which have been used to determine the transfer of substances across plant protoplasts and also to measure their permeability. [For particulars and literature references, see (34).] But regrettably most of these methods are subject to sources of error which in most cases will render the results of such measurements more or less inaccurate and unreliable. One such quite general source of error has already been referred to, viz., the fact that the diffusion resistance of the cell wall and of the cell sap cannot be readily distinguished from that of the protoplasm, which thus seems greater than it really is. Especially in the case of rapidly penetrating substances such as, say, water, the errors caused in this way may be very great. Another point of general importance is the danger that the cells studied may have been damaged in some way so that their permeability has been pathologically increased or their power of active transport reduced. The gentlest possible handling of the cells is therefore necessary in carrying out such measurements.

The methods to be discussed will be grouped under five main headings: (*1*) Methods Involving Visible Changes within the Cells, (*2*) Analytical Methods, (*3*) Osmotic Methods, (*4*) Electrical Methods, and (*5*) Methods Based on Physiological Toxicological Effects.

1. Methods Involving Visible Changes within the Cells

a. Vital staining. Investigations into the permeability of living cells to dyes may seem very promising, for the penetration of dyes is often

directly observable under the microscope. No wonder, therefore, that vital staining experiments have gained great popularity. In reality, however, as we shall see later on, the estimation of cell permeability on the basis of such experiments is far from easy. It is therefore really a pity that so many permeability studies have been based on this particular method.

b. Color changes of indicators. Acids and bases may produce color changes within cells containing either natural indicators (anthocyanins) or artificial ones (e.g., neutral red). It is, however, not easy to obtain quantitative results in this way, owing to the extent to which the cell sap may be buffered.

c. Precipitation method. Caffeine and other alkaloids, even in very low concentrations, cause precipitates within cell saps containing tannins. Up to now this method of studying permeability has only been used in a semiquantitative way.

All the above methods involve mechanisms of storage or binding of the solute after entry to a degree which affects the interpretation of the permeability per se.

2. *Analytical Methods*

In recent times direct analytical methods have been increasingly used for permeation studies. After immersion of cells or tissues in solutions of known composition, one can make an analysis either of the cells (or tissues) themselves, of the sap isolated from the cells, or of the immersion medium. The accuracy and reliability of the results obtained depend very much upon the nature of the experimental objects used.

Giant coenocytic vesicles, large enough to be handled singly, yield the most unambiguous results. Examples of the marine algae *Valonia* and *Halicystis* are described in this volume, Chapter 4. The isolation of samples of the sap from such cells is very simple. Even a perfusion of the living cell with solutions of known composition is possible. However, for many purposes the considerably smaller but more easily obtainable internodal cells of different members of the Characeae (*Nitella*, *Chara*, etc.) are equally suitable. In view of the slowness of diffusion it is often even an advantage that these cells, which generally have a thickness of about 0.2–1 mm and a length of about 2–10 cm, are not as bulky as the vesicles of *Valonia*. Moreover, the content of a single *Nitella* cell may well be enough for accurate microchemical determination. Sometimes it may be found most appropriate first to "saturate" the cell in a solution of the substance to be tested and then to follow its gradual outflux after the cell has been rinsed and transfered into pure water.

While a certain amount of work has been done with suspensions of bacteria, yeasts, or unicellular algae, the study of the permeability properties of such organisms is still more or less in its beginnings (see this volume, Chapter 4 for active transport in these organisms).

Cut disks of fleshy storage organs such as potato (*Solanum tuberosum*) tubers, beet roots, carrot (*Daucus carota* var. *sativa*) roots, etc., have been very extensively used for studies of both the loss and the absorption of solutes. The results, however, obtained with these systems relate more closely to active transport than to passive permeability and they are fully treated in this volume, Chapter 4. The same applies to excised roots and studies on various water plants.

Besides chemical analysis, several physical methods (spectroscopic, polarographic, conductrometric) have also been used for estimation of the often very small quantities of different substances taken up, or given off, by cells or tissues. In this connection the radioactive isotopes or tracers, of which a large assortment (e.g., C^{14}, Na^{24}, P^{32}, S^{35}, Cl^{36}, K^{42}, Ca^{45}, Fe^{55}, Zn^{65}, Br^{82}) is now available, deserve special mention. Their use in permeation studies is of great importance not only because the tracers can be determined, for instance with a Geiger counter, in extremely low concentrations, very exactly and yet comparatively easily. Still more important is the fact that the use of tracers enables us to follow the fate of certain atoms, ions, or molecules irrespective of the presence of other atoms, ions, or molecules of the same species. Thus a study of the exchange of, say, potassium ions against potassium ions, or of chlorine ions against chlorine ions, has been made possible by this method. Likewise and for the first time, it has proved possible to measure in a convincing manner the permeation power of single ionic species. Among the nonradioactive isotopes, H^2, N^{15}, and O^{18} are of greatest interest to the physiologist. Information concerning the isotope technique may be found in several handbooks (5, 37, 101a, 136, 138a, 148, 165).

3. *Osmotic Methods*

A feature common to the osmotic methods is that the entrance or exit of substances is not observed directly but only inferred from the consequential movement of water. The correctness of the osmotic permeation determinations thus rests on certain assumptions, the most important of which are the following: (a) the exchange of water between a cell and its bathing fluid is controlled practically solely by osmotic forces, not by imbibition phenomena, active water transport, etc. (b) Solutes taken up by the cells keep their osmotic water-retaining power unchanged in the cell. (c) No exosmosis of normal cell constituents

occurs during the experiment, nor is the osmotic value of the cell sap liable to changes due to metabolic processes.

General statements as to the justification of these assumptions are at present difficult to make, since the literature contains very conflicting evidence. Probably different types of cells behave very differently in this respect. Each case therefore requires appropriate control experiments.

In spite of this theoretical unreliability of the osmotic methods, they have played, and still play, a very important role in the exploration of the permeability phenomena. They are, in fact, of very general applicability and are among the most convenient to use. They may be divided into two groups: plasmolytic and nonplasmolytic methods.

The method of incipient (or, more properly, limiting) plasmolysis (*Grenzplasmolyse*) has been used by Overton (125), Fitting (58), Bärlund (8), and many others. Cells with osmotic concentrations as nearly equal as possible are put into a graded series of solutions, of increasing concentration, of the substance whose permeation is to be followed. The cells are then examined from time to time as to the appearance and disappearance of plasmolysis. Suppose that at a certain time the faintest traces of plasmolysis are observed in a 0.20 *M* solution, while 1 hour later the same state of limiting plasmolysis is found in a 0.25 *M* solution. In such a case it seems plausible to assume that during the 1 hour enough solute has entered the cells to raise the concentration of their sap from the equivalent of 0.20 *M* to 0.25 *M*. If the substance to be studied is so poorly soluble that a saturated solution of it does not cause any plasmolysis, it may be applied together with some nonpermeating substance (e.g., sugar or mannitol), the concentration of the latter being such that it would not alone plasmolyze the cells. This "method of partial pressures" is also applicable in the case of substances permeating so rapidly that they would not alone bring about a recognizable plasmolysis.

The plasmometric method of Höfler (78) has also proved very useful. In this case the cell to be studied is thoroughly plasmolyzed in a fairly concentrated solution of the substance to be tested. The volume of its protoplast is then evaluated from time to time by microscopic measurements. The more rapidly its volume is found to increase, the more rapidly the solute has entered the protoplast. This method requires only a single cell, but both the cell and its plasmolyzed protoplast should have geometrically simple forms to facilitate exact volume calculations.

If one wishes to compare the permeation powers of two substances, *A* and *B*, under strictly identical conditions, this can easily be done by a special modification of the plasmometric method. The cell is first

plasmolyzed in an m molar solution of a nonpermeating sugar where the protoplast assumes the volume v. It is then transferred to a solution m molar as to sugar and n molar as to A. Here it is left until the concentration of A inside the protoplast has reached the same value as that outside it, as can be seen from the fact that its volume is again v. The external solution is now replaced by a solution which is m molar as to sugar and n molar as to B. Under these conditions A will diffuse out from the cell while B is at the same time diffusing in the opposite direction. Three alternatives are now conceivable: (a) $P_A = P_B$. The volume of the protoplast remains constant. (b) $P_A < P_B$. The volume first increases and then becomes equal to v. (c) $P_A > P_B$. The volume first decreases and then becomes equal to v.

When the protoplast shrinks away from the cell wall in plasmolysis, the original plasma membrane is probably often destroyed and a new osmotic membrane is formed. It would therefore not be surprising if the permeability of plasmolyzed protoplasts differed greatly from that of nonplasmolyzed ones. Experiments by Schmidt (149) and others have shown, however, that the difference in permeability is in most cases fairly slight. At any rate, it is fortunate that there are also some nonplasmolytic osmotic methods of permeability measurement. Thus, the changes in cell volume caused by osmotic withdrawal or uptake of water may be observed by watching the changes in length either of single cells or of tissue strips. Moreover, in *Beggiatoa mirabilis* and *Oscillatoria* spp. the withdrawal of water from the cells causes an easily observable inward bending (*Knickung*) of the lateral cell walls. Other nonplasmolytic osmotic methods have recently been described (102, 167).

4. *Electrical Methods*

The permeability of cells to ions may be studied by measuring the electrical conductivity of whole tissues or single cells. The first alternative is scarcely to be recommended, however, since it is difficult to ascertain to what extent the electric current passes through the protoplasts and to what extent it goes through the cell walls around the protoplasts. Analogous experiments with single cells of *Valonia, Nitella*, etc., are in this respect more reliable. It is even possible to insert an electrode into one such giant cell and then to measure directly the resistance across the protoplast.

5. *Methods Based on Physiological or Toxicological Effects*

Suppose it is found that a solution of a certain acid, A, rapidly kills cells of a given kind, while an equimolar solution of another acid, B, under the same conditions only exhibits harmful effects against cells

of the same type after a longer time. This result might be explained by assuming that *B* does not enter the cells as rapidly as *A* does. But, of course, such a conclusion requires a rigorous examination of all the experimental circumstances before being definitely adopted. Moreover, truly quantitative permeability determinations are not easily attained in this way. However, experiments of this type are of special interest in so far as they may supply knowledge concerning the permeability of the outer plasma membrane, whose permeability properties are still very imperfectly known.

VI. Sites of Resistance to Penetration

A. Localization of Penetration Resistance in the Protoplast

Is penetration resistance uniform throughout the whole protoplast, or are there some zones of higher, and others of lower, resistance? This question was first put, and also answered, by Pfeffer (130, 132), who advanced the hypothesis that the resistance to permeation is principally due to two extremely thin plasma membranes, the one covering the outer surface of the protoplast, the other separating the cell sap from the protoplasm. The outer plasma membrane is now generally called the *plasmalemma* [Plowe (133)], while the inner one is called the *tonoplast* [de Vries (169)]. The bulk protoplasm between them is the *mesoplasm* (133).

The existence of an outer plasma membrane was postulated by Pfeffer on the basis of the following observation, among others. By treating protoplasts with dilute acid solutions it is possible to cause the outermost layers to assume a condition of rigor without destroying their initial impermeability toward certain dyes. Only after the outermost layer is ruptured will the dye enter through the tear and then rapidly spread throughout the interior of the protoplast.

The existence of an inner plasma membrane was first clearly demonstrated by de Vries (169), who was able to kill the bulk of the protoplast in such a way that its innermost layer still displayed the permeability properties of the intact protoplast virtually unchanged.

De Vries originally assumed that every plasma membrane was derived from a previous plasma membrane, just as a nucleus always originates from a previous one. Pfeffer demonstrated, however, that plasma membranes can form *de novo* when an algal filament is crushed in water, for the extruded spheres of protoplasm possess similar osmotic properties to the original cell. It is thus logical to conclude that a new plasma membrane is at once produced when the inner parts of the protoplasm are brought into direct contact with water. The following

experiment of Pfeffer (132) is also very instructive. Small crystals of asparagine are introduced into the body of a myxomycete plasmodium immersed in a saturated solution of asparagine in water. If the bathing fluid is now diluted, the crystals imbedded in the protoplasm will dissolve in the water imbibed by the protoplasm. While this process is going on, sharply defined vacuoles form around the gradually vanishing crystals. These *de novo* produced "vacuoles" are found to behave osmotically just like natural ones.

The introduction of microinjection methods some forty years ago enabled new, and very fruitful, approaches to this problem. Thus Chambers made some impressive experiments consisting of the injection of ammonium chloride or sodium bicarbonate solutions into starfish eggs stained with neutral red. Jacobs (94) gives the following vivid descriptions of them: "Eggs were first stained and placed for a few moments in the ammonium chloride until the color of the intracellular indicator had visibly changed in the alkaline direction. Some of the same ammonium chloride solution was then injected into an individual egg. Immediately the indicator assumed its acid color at the point of injection and, what is more important, there was a rapid spread of the acid condition in all directions from the point of injection, which did not cease until the boundary of the egg had been reached. The last region of the egg in which the change from alkalinity to acidity occurred was therefore that which from the beginning of the experiment had been almost in contact with the solution in question. Experiments with the carbon dioxide-bicarbonate buffer system gave results which were entirely the same in principle except that the conditions of acidity and alkalinity were the reverse of those just described."

With plant cells, microinjection experiments are not so easily performed owing to the rigid cellulose wall which envelops the protoplast. Nevertheless, some very significant results have been achieved. The most important experiments were those of Plowe (133) concerning the behavior of certain dyes when injected into root hairs of *Trianea bogotensis*, epidermal cells of *Allium cepa*, and cells of the red alga *Griffithsia bornetiana*. All the dyes tested (Aniline Blue, Acid Fuchsin, bromocresol purple, phenol red) were such that they did not enter the cells when these were immersed in the dye solution. When injected into the central vacuole they spread in it but did not penetrate the protoplast. On the other hand, when injected into the mesoplasm itself, they were able to spread in the protoplast but unable to pass through the tonoplast into the vacuole or through the plasmalemma into the external solution. (In view of the fundamental importance of these experiments a repetition and extension of them seems desirable.)

These observations show that plasma membranes do exist and that they are less permeable, at least toward certain substances, than is the mesoplasm. There are still, however, quantitative problems to be solved. For, as Höfler (77) pointed out, if we put

$$R = R_L + R_M + R_T$$

where R denotes the permeation resistance of the whole protoplast, while R_L, R_M, and R_T give the resistances of plasmalemma, mesoplasm, and tonoplast, respectively, then it will of course be of great importance to designate the relative magnitudes of the three terms in question.

Let us begin with the permeation resistance of the mesoplasm. If, as is generally assumed, it contains plenty of both free and bound water, so that a coherent aqueous phase extends across the whole mesoplasm from the tonoplast to the plasmalemma, then it seems rather difficult to imagine that even the passage of strongly hydrophilic substances through the mesoplasm will be impeded much more than their diffusion through a water layer of corresponding thickness. Only in the case of extremely rapidly penetrating substances, such as water, might the diffusion resistance of the mesoplasm be of the same order of magnitude as that of the plasma membranes.

We now come to a more difficult question: How great is the penetration resistance of the plasmalemma as compared with that of the tonoplast?

In recent times it has often been claimed that the resistance of the plasmalemma is very much lower than that of the tonoplast and that many substances will therefore readily enter the bulk protoplasm from the ambient solution, although they do not reach the cell sap (3, 77). Or, as Höfler puts it: the "intrability" of the protoplast may be considerable, although its permeability is very low. Several quite different arguments have been advanced in favor of such views.

First, it is well known that the protoplast as a whole is almost impermeable to sugars, amino acids, and mineral salts—in fact, to many substances which play an important role in the metabolism of every cell. Now, it has often been thought that this paradox can be understood only if we assume that the great penetration resistance to substances of vital importance is located principally in the tonoplast alone, the plasmalemma being more or less freely permeable to the substances in question. It seems, however that those reasoning in this way have overlooked the fundamental fact that the uptake of substances into living protoplasts is not due to simple diffusion processes alone but is due, to a very large extent indeed, to active transport. In fact, it seems very probable that the plasmalemma is a site of effective transport activity,

but this, of course, does not imply that its permeability must also be great.

Among the more direct observations which have been thought to indicate a relatively high permeability of the plasmalemma are those of Höfler (77, 80; cf. 153) on cap plasmolysis (*Kappenplasmolyse*). He found that the protoplasts of certain cells, when plasmolyzed in pure solutions of alkali chlorides, will sometimes swell considerably at their ends, thus indicating the fairly rapid entrance of the salt into the mesoplasm, although it does not enter the vacuole, which still remains strongly contracted. This is, no doubt, a very interesting phenomenon. On the other hand it occurs only occasionally, and so it does not prove that the plasmalemma is always, or even often, considerably more permeable to ions than is the tonoplast.

Moreover, according to Brooks (24) radioactive phosphate, sodium, and potassium ions are taken up considerably more rapidly into the protoplasm of *Nitella* cells than into their sap.

Finally, the observations concerning the so-called "apparent free space" in plant tissues have attracted great attention. By apparent free space is meant that portion of a tissue to which solutes apparently move "by free diffusion," i.e., without encountering any considerable resistance. In part, of course, this space, consists of cell walls and intercellular spaces, but according to the calculations of several recent investigators it is necessary to assume that the protoplasm, or at least parts of it, also belong to the apparent free space. If this assumption were correct, then it would follow that the plasmalemma must be readily permeable to ions, which only penetrate the tonoplast very slowly. As can be seen, for instance, from recent reports by Epstein (54, 55), Kramer (107), and Robertson (140), there are, in fact, several observations which appear to sustain such views. But, on the other hand, it may be permissible to doubt whether the evidence so far presented in favor of these ideas is convincing enough—to be a basis for such far-reaching conclusions as these, which would place the inclusions of the cytoplasm in virtual free contact with the changing composition of the external solution [cf. Levitt (110a)].

Such doubts seem the more justified when we remember the strong evidence suggesting that the diffusion resistance of the plasmalemma is of considerable magnitude. First, we have the analogy with the animal cells, in which the great diffusion resistance of the outer plasma membrane is in many cases extremely well established. Secondly, there is the general view that a condition for the undisturbed course of the life processes is that the living machinery should be effectively isolated from its environment, which, especially in the case of many unicellular

organisms, may be subject to sudden changes in chemical composition. This requirement seems, in fact, to be well met, most protoplasts being remarkably resistant to lipid-insoluble poisons which, if they were to gain access to the interior of the protoplasts, could scarcely fail seriously to disturb the life processes. Thus, for instance, it is known that even wide fluctuations of the pH of the medium scarcely influence the rate of oxygen consumption of yeast. Moreover, Conway and Downey (40) have directly demonstrated that more or less lipid-insoluble acids (e.g., glyceric, malic, citric, and tartaric acid) can readily penetrate only a very restricted part of the yeast cell, the accessible region being probably identical with the cell wall alone. The plasmalemma of the cells of land plants may, on the whole, be somewhat more delicate (83), but in most cases, nevertheless, their protoplasts, too, show a fairly high degree of resistance to lipid-insoluble poisons, thus indicating that these substances can have almost no access to the mesoplasm across the intact plasmalemma. Finally, we have to remember that when plant cells are put into hypertonic solutions the protoplast, in the vast majority of cases, will contract as a whole, while, if the tonoplast alone were endowed with semipermeability, one would expect that only the vacuole would contract.

The problem of the permeability properties of the plasmalemma is thus, at present, rather puzzling in that suggestive arguments have been put forth in favor of, and also against, the existence of a great resistance to diffusion at the outer surface of the protoplast. The present writer inclines to the classical view, according to which the plasmalemma resembles the tonoplast in its diffusion resistance. But evidently it is not possible yet to answer this question definitely, least of all in a really quantitative manner. There is, of course, also the possibility that the location of the main osmotic barrier may vary in different kinds of cells and according to the solutes in question.

At all events, the present uncertainty concerning the permeability of the plasmalemma is one of the most serious gaps in our knowledge of the permeability properties of plant cells, because, for the functioning of the living machinery it must be far from insignificant whether the osmotic barrier is situated on the outer or the inner surface of the protoplasmic layer.

Concerning the tonoplast membrane, there seems to be fairly general agreement that its diffusion resistance toward hydrophilic substances is great. But here, too, reliable quantitative data are almost totally lacking.

A very special case is represented by the sieve tubes and latex vessels, which are believed to be devoid of tonoplasts [(60), page 84]. The same, of course, holds true of all cells lacking vacuoles.

B. PENETRATION RESISTANCE OF THE CELL WALL

On its way from the medium into the cell sap, or vice versa, a permeating molecule or ion has to overcome not only the resistance of the protoplast, including its membranes, but also that of the cell wall.

It is well known that the permeability of cutinized or suberized cell walls, even to water, is very restricted. On the other hand, strongly hydrophilic walls composed only of building materials such as cellulose, hemicelluloses, and pectins are readily permeable to the great majority of water-soluble substances. Nevertheless, such a wall represents an unstirred layer that by virtue of its thickness alone will offer a certain

TABLE II

PERMEABILITY OF THE CELL WALL OF *Nitella mucronata* TO NONELECTROLYTES[a,b]

Substances	M[c]	$P \times 10^5$	$PM^{1/2} \times 10^4$
Methanol	32	75	43
Ethylene glycol	62	56	44
Glycerol	92	44	42
Tetraethylene glycol dimethyl ether	222	24	36
Sucrose	342	17	31
"Polyethylene glycol 400"	400	7.6	15
Raffinose	504	8.3	19
"Polyethylene glycol 600"	600	2.7	7
"Polyethylene glycol 1000"	1000	0.8	3

[a] The permeability constants (P) are given in centimeters per second, multiplied by 10^5.

[b] According to Collander (32) and some supplementary unpublished determinations.

[c] M = molecular weight.

resistance to diffusing molecules, which is more important as the diffusion resistance of the protoplast becomes smaller. At the same time, such a hydrophilic cell wall will function as a molecular sieve more or less impervious to all molecules whose molecular size exceeds a certain limit. As an example, the cell wall of *Nitella mucronata* may be taken (Table II). Although the values of the permeation constant stated in the table have not a high degree of accuracy, taken as a whole they show a fairly regular decrease of the permeation power with increasing molecular size. Moreover, the constancy of the $PM^{1/2}$ values at the beginning of the series and their rapid decrease at the end of it indicates how the sieve action of this cell wall gradually exerts its effect once the molecular weight exceeds a value of about 200 or 300. Finally, it should perhaps be pointed out that, although the permeability of the

wall to such large-molecular substances as "polyethylene glycol 1000" may, at first sight, appear very small, it is nevertheless roughly 1000 times greater than the permeability of the protoplasts of *Nitella* toward, say, glycerol. Generally speaking, then, the hydrophilic cell walls will very seldom be an important hinderance to the uptake of solutes into plant cells.

For further particulars concerning the permeability of plant cell walls, the reader is referred to Brauner (21).

VII. Permeability to Nonelectrolytes

A survey of the permeability of cells to various substances may appropriately begin with nonelectrolytes. Members of this group are free from the complications which arise from variable ionization of the permeators, and the great variety of structure shown by these substances renders them especially suitable for studies of the relations between chemical and physical properties, on the one hand, and permeation power, on the other. Finally, the permeability of plant cells to nonelectrolytes may be said to be decidedly better known at present than their permeability to electrolytes.

In the following, we shall designate as nonelectrolytes all those substances which, at physiologically tolerable pH values, occur almost solely as nonionized molecules. Under the heading of nonelectrolytes we will thus also treat such extremely weak bases as urea, caffeine, and antipyrene, all of which have dissociation constants below 10^{-10}. Even the amino acids may conveniently be taken into consideration in this context inasmuch as they have no net electric charge.

A. OVERTON'S GENERAL PERMEABILITY RULES

As already stated, Overton, after studying the behavior of a great number of plant and animal cells toward several hundreds of chemical compounds, reached the conclusion that some general rules govern the relative permeation power of substances toward extremely different types of cells. These rules, which on the whole have been corroborated by later investigators, may be summarized as follows:

(1) Hydrocarbons, their halogen and nitro derivatives are all extremely lipophilic and have a very great permeation power.

(2) An increase in the number of hydroxyl groups tends simultaneously to decrease the lipid solubility and the permeation power. Thus, the monohydric alcohols permeate very rapidly, the dihydric ones considerably more slowly but still fairly rapidly, glycerol (a trihydric alcohol) rather slowly, erythritol (a tetrahydric alcohol) still more slowly. Finally, the hexahydric alcohols (e.g., mannitol), the

hexoses, and the corresponding di-and trisaccharides all have a scarcely detectable permeation power.

(3) A C=O group has qualitatively the same effect on the distribution and on the permeation as a C—OH group.

(4) A similar but stronger effect is exerted by the amino and especially by the $-CONH_2$ group. Thus the amides of the monobasic acids (e.g., acetamide) have about the same permeation power as the dihydric alcohols. Urea permeates rather slowly.

(5) Amino acids have an extremely low lipid solubility and permeate extremely slowly.

(6) An increase in the length of the carbon chain increases the lipid solubility and the permeation power. A similar effect is also obtained by substitution of the hydrogen atoms in the hydroxyl, carboxyl, or amino groups by alkyl, aryl, or acyl groups. Thus the permeation power increases strongly in the series glycerol < monoacetin < diacetin < triacetin and also in the series urea < methylurea < ethylurea < diethylurea < triethylurea.

(7) Substitution of oxygen atoms by sulfur atoms increases lipid solubility and permeation power. Thus, thiourea permeates somewhat more readily than does urea.

Overton claimed that these rules are valid for all kinds of cells. We shall see, however, that a few incontestable exceptions occur.

B. Lipid Solubility, Polarity, Hydrophilia, Hydrogen-Bonding Tendency

In view of the correlation found by Overton, and also by later investigators, between lipid solubility and permeation power the question of the relation of lipid solubility to other physicochemical properties and to the chemical constitution arises. Of course, when we are here speaking of lipid solubility, the relative lipid solubility alone is meant, i.e., the distribution coefficient lipid: water.

Little is actually known about the solvent properties of those lipids (phosphatides, sterols, etc.) which are commonly supposed to occur in the plasma membranes and whose solvent properties are therefore most relevant. It therefore seems best to consider the solvent properties of water-immiscible organic solvents in general: since these have so much in common, we shall be able in this way to conclude, indirectly, a great deal concerning the solvent properties of those more or less unknown cell lipoids in which we are primarily interested.

First, however, just a few words concerning two pairs of terms which are often used in this connection, namely, the terms polar and nonpolar, hydrophilic and hydrophobic. Polar compounds are character-

ized by a molecular structure which involves a shifting of one or more electrons from their original position in the atom in such a way that the molecule will show regions of positive or negative electrical charge. The hydroxyl, carboxyl, and amino groups may be mentioned as examples of polar groups. The nonpolar compounds, on the other hand, of which the hydrocarbons are the most typical representatives, have a molecular structure which results from the sharing of electrons by the atoms concerned without the production of regions of great electrical dissimilarity. Now, on the whole, polar compounds tend to be more soluble in water (which is itself highly polar) than in such nonpolar or weakly polar organic solvents as hydrocarbons, ether, etc. Nonpolar substances show the reverse relation.

Substances preferentially soluble in water are also called hydrophilic, while those preferentially soluble in organic solvents are called hydrophobic,[4] lipophilic, or organophilic. The essential difference in nature between these two groups of substances was virtually elucidated some twenty years ago when the concept of hydrogen bonds (or hydrogen bridges, as they are sometimes called) was advanced. It had for a long time been known that water is in many respects a very peculiar liquid (cf. 56). Thus, its vaporization heat, its surface tension, and its cohesion are unusually great. Furthermore, its boiling point is surprisingly high, as is at once realized by comparing it with that of the closest analog of water, namely, hydrogen sulfide, which has a boiling point of —60°C. These anomalies of water are understandable if we take into account that the H_2O molecules in liquid water are not free but are bound by hydrogen bonds into netlike complexes. Generally speaking, the hydrogen bond is a chemical bond resulting from the attraction of two electronegative atoms for a hydrogen atom. Only the most strongly electronegative atoms are capable of forming hydrogen bonds, namely, oxygen, nitrogen, chlorine, and fluorine atoms. Now, the hydrophilia is just a manifestation of the tendency of a substance to form hydrogen bonds: thanks to its hydroxyl, carboxyl, amino, or other similar groups, it is able to form hydrogen bonds with the water molecules and thus at the same time attracts, and is itself attracted by, water molecules. On the other hand, molecules not capable of forming hydrogen bonds are, as it were, squeezed out from water and other strongly hydrogen-bonded liquids and thus they tend to accumulate in a neighboring nonaqueous liquid.

Now, it is the degree of hydrophilia, or hydrophobia, of the solutes

[4] Strictly speaking there exists no hydrophobia but only an absence of hydrophilia, just as darkness is nothing but the absence of light. In many contexts the word hydrophobia is nevertheless quite useful.

on the one hand and of the solvents on the other that is the main factors which governs the distribution of solutes between water and water-immiscible solvents. Here the old rule *similia similibus solvuntur* holds true. Thus, the more hydrophilic the solute and the more hydrophobic the nonaqueous solvent, the more completely the solute will accumulate in the aqueous phase. For instance, in the series propanol, propylene glycol, glycerol, the distribution coefficient in the solvent system ethyl ether-water falls off rapidly with increasing number of hydrophilic hydroxyl groups: $1.9 > 0.018 > 0.0007$. In the solvent system hexane-water, owing to the more strongly hydrophobic nature of the hexane, the distribution coefficients of the same substances are lower and fall off still more steeply, something like this: $0.04 > 0.0001 > 0.000001$.

Along with the hydrophilia yet another factor, namely, the acidity or basicity of solutes and solvents, may influence the distribution. But here the rule holds true that opposites attract each other, that is, acidic solutes tend to accumulate in basic solvents, while basic solutes have a stronger affinity for acidic solvents. In fact, even very low degrees of acidity or basicity may markedly influence the distribution. Thus, for instance, fatty acid amides have an appreciably greater affinity for alcohols than for ethers of a corresponding degree of hydrophilia.

Except for these two major factors, namely, the hydrophilia-hydrophobia and the acidity-basicity factors, the distribution is scarcely influenced by other more specific properties of the solvents. Thus, in spite of the many organic, water-immiscible solvents, they can all be classified into a consistent, readily surveyed system on the basis of these two criteria, viz., their varying hydrogen-bonding tendency, on the one hand, and their acidity or basicity, on the other. From the standpoint of cell permeability this has a very important practical consequence: Although almost nothing has so far been directly ascertained concerning the solvent properties of those lipids (phosphatides, sterols) which are most commonly supposed to occur in the plasma membranes, we are nevertheless in the position to predict, in a general way, the limits within which their solvent properties will vary.

C. PERMEABILITY OF THE INTERNODAL CELLS OF THE CHARACEAE

The results of Overton were published, as we have seen, in a semi-quantitative form only. We will now turn to later, more truly quantitative, permeability measurements. In doing so, we will begin with the internodal cells of two characean plants, *Chara ceratophylla* (35) and *Nitella mucronata* (32), since these cells have been more thoroughly studied as regards their permeability to nonelectrolytes than have other types of plant cells. Moreover, the method of permeability measure-

ment used in these studies was comparatively reliable, since it was based on quantitative microchemical determinations of the actual amounts of substances entering the cells, or leaking out from them, during appropriately chosen time intervals.

The authors of these works were anxious to decide to what extent the observed transfer of solutes may be regarded as due to simple permeation processes and to what extent it may be due to active transport. To this end they first studied the concentration of the permeators in the cell sap after equilibrium had been reached. They found that in the cases studied the equilibrium concentration in the cell sap was about 90–100% of the concentration in the medium. Furthermore, the general course of the uptake was found to agree fairly with that predicted for simple diffusion process. Finally, the transfer rates were found to be the same whether the solutes were entering the cells from outside or, on the contrary, were moving from the cell sap into the bathing fluid. All these findings strongly suggest that the transfer processes so studied were not influenced appreciably by metabolism or by chemical combination of the permeators with any cell constituents. (This conclusion has been disputed by Bogen (16), but his arguments do not seem convincing.)

Figure 1 presents a survey of the principal results achieved with the *Nitella* cells and of their bearing on current permeability theories. To this end the permeability constants, P, multiplied by the molecular weights, M, raised to the power 1.5 have been plotted against the distribution coefficients in the system olive oil-water. The magnitude $PM^{1.5}$ has been chosen rather than P in order that the points which represent the different permeators shall fit a straight line as closely as possible. Besides, among the substances studied the variation in the magnitude of M is so slight (from 19 to 480) that, say, 1.2 or 1.8 used instead of 1.5 as exponent of M would not alter the picture very much. On the other hand, it will be noted that while the points representing molecules of medium or comparatively great molecular weight ($M = 60$–120 or 120–480, respectively) are fairly evenly distributed around the midline of Fig. 1, the smallest molecules ($M < 60$) are all situated above that line. Finally, it will be observed that the mid-line has a slope of about 53° to the abscissa. We see, then, that for molecules of a molecular weight between 60 and 480 the permeation constant P is roughly proportional to the expression $k^{1.5}/M^{1.5}$ (where k denotes the distribution coefficient olive oil:water) but the smallest molecules permeate somewhat more readily than this formula implies.

With the cells of *Chara* and of a third characean plant, *Nitellopsis obtusulus*, virtually similar results were obtained, except that the

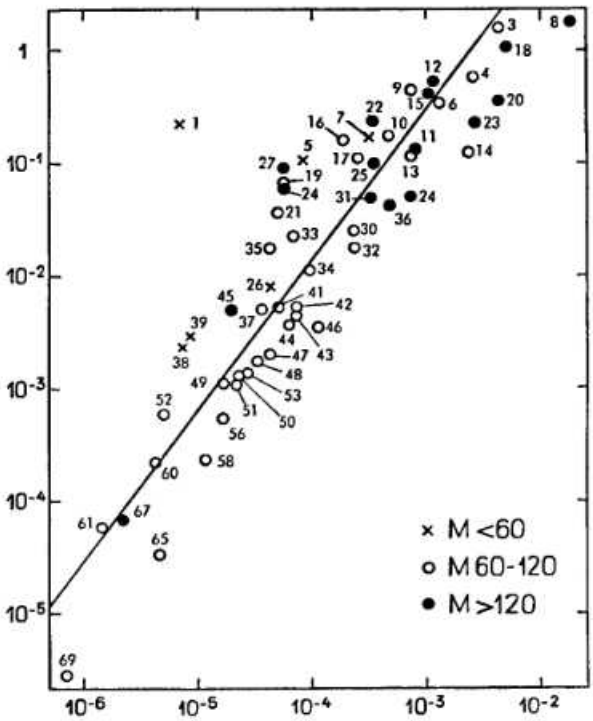

FIG. 1. Correlation between the permeation power of several nonelectrolytes toward *Nitella mucronata* cells on the one hand and their relative oil solubility and molecular weight on the other. Ordinate: $PM^{1.5}$; abscissa: distribution coefficient olive oil:water. The substances are: *1*, HDO; *3*, methyl acetate; *4*, *sec*-butanol; *5*, methanol; *6*, *n*-propanol; *7*, ethanol; *8*, paraldehyde; *9*, urethane; *10*, isopropanol; *11*, acetonylacetone; *12*, diethylene glycol monobutyl ether; *13*, dimethyl cyanamide; *14*, *tert*-butanol; *15*, glycerol diethyl ether; *16*, ethoxyethanol; *17*, methyl carbamate; *18*, triethyl citrate; *19*, methoxyethanol; *20*, triacetin; *21*, dimethylformamide; *22*, triethylene glycol diacetate; *23*, pyramidon; *24*, diethylene glycol monoethyl ether; *25*, caffeine; *26*, cyanamide; *27*, tetraethylene glycol dimethyl ether; *30*, methylpentanediol; *31*, antipyrene; *32*, isovaleramide; *33*, 1,6-hexanediol; *34*, *n*-butyramide; *35*, diethylene glycol monomethyl ether; *36*, trimethyl citrate; *37*, propionamide; *38*, formamide; *39*, acetamide; *41*, succinimide; *42*, glycerol monoethyl ether; *43*, *N,N*-diethylurea; *44*, 1,5-pentanediol; *45*, dipropylene glycol; *46*, glycerol monochlorohydrin; *47*, 1,3-butanediol; *48*, 2,3-butanediol; *49*, 1,2-propanediol; *50*, *N,N*-dimethylurea; *51*, 1,4-butanediol; *52*, ethylene glycol; *53*, glycerol monomethyl ether; *56*, ethylurea; *58*, thiourea; *60*, methylurea; *61*, urea; *65*, dicyanodiamide; *67*, hexamethylenetetraamine; *69*, glycerol. From Collander (32).

values of P were found to be more closely proportional to $k^{1.0}/M^{1.5}$ in the case of *Chara* and to $k^{1.15}/M^{1.5}$ in the case of *Nitellopsis*. In these cases, too, molecules of a molecular weight below 60 were found to have a greater permeation power than the expressions given would imply.

If $PM^{1.5}$ is plotted against the distribution coefficient ether:water instead of olive oil:water, the resulting graph will be fairly similar to Fig. 1, with the exception, however, that the points representing slightly basic substances (amines, amides) will be found lying too low. In this respect, then, olive oil, probably owing to the free oleic acid which it contains, is a decidedly better model substance than ether. Besides, by adding more oleic acid to the olive oil its solvent capacity toward slightly basic substances can be considerably increased.

The theoretical significance of these empirical results will be discussed below (Section XIII).

D. Different Permeability Types

As already stated, Overton very strongly stressed the essential similarity, as to their permeability properties, between protoplasts of widely different origin, shape, and function. He did not fail to observe that there are also minor differences in this respect between different cells, but for reasons readily understood these differences did not interest him as much as the striking and unexpected resemblances between otherwise different cells. It was therefore only about 1930 that systematic investigations were started in order to reveal and, if possible, explain specific differences in permeability.

In the field of animal cells, such investigations have been successfully carried out by Jacobs (95, 98), who studied the permeability properties of the erythrocytes in different animal groups, detecting numerous interesting parallels between specific permeability properties, on the one hand, the taxonomic classification, on the other. These results are the more striking since they refer to cells with one and the same physiological function.

In the botanical field, "comparative" permeability studies were inaugurated in 1930 by Höfler (85a, 90b), who stressed the importance of establishing and comparing specific permeability series (*spezifische Permeabilitätsreihen*) for different plant protoplasts. In the following years his program was carried out by numerous workers, among whom Hofmeister (86), Marklund (112), and Elo (52) may be named. The results of these studies have been summarized by Höfler (82), who distinguished the following five permeability types.

Type I. The first type is called, by Höfler, the *Chara-Maianthemum*

Type. This denomination is derived from the names of two test objects, viz., the internodal cells of *Chara ceratophylla* and the subepidermal cells of the stem of *Maianthemum bifolium* [studied by Höfler (78)], which are considered to be characteristic of this type. The type might equally, however, be called the Main or Normal Type, for as a matter of fact the majority of plant protoplasts so far studied are of this type.

Table III gives a few, rather arbitrarily chosen examples illustrating the behavior of the representatives of this group. In spite of their very different nature (*Maianthemum* is a terrestrial flowering plant; *Chara*, a highly specialized green alga from brackish water; *Pylaiella litoralis*,

TABLE III

PERMEABILITY OF *Chara ceratophylla*, *Maianthemum bifolium*, AND *Pylaiella litoralis* CELLS TO SOME NONELECTROLYTES[a]

Substance	*Chara*[b]	*Maianthemum*[c]	*Pylaiella*[d]
Ethylene glycol	58	—	106
Thiourea	10	9.5	—
Methylurea	9.2	8.0	14
Lactamide	7.5	3.4	—
Urea	5.4	2.8	2.8
Glycerol	1.0	1.0	1.0
Malonamide	0.19	0.56	0.67
Erythritol	0.06	0.08	0.04

[a] The permeability to glycerol put equal to unity.
[b] According to Collander and Bärlund (35).
[c] According to Höfler (78).
[d] According to Marklund (112).

a marine brown alga) the similarity in their permeability is unmistakable.

Type II. The *Gentiana-Sturmiana* Type of Höfler is characterized by its unusually high permeability toward urea; urea penetrates these protoplasts more rapidly than does methylurea, while in "normal" cases the permeation rate of methylurea is about 2–5 times as great as that of urea. The last-mentioned behavior can be explained because the lipoid solubility of methylurea is some 2–5 times as great as that of urea. The more rapid permeation of urea, on the other hand, is probably due to some special feature, not yet exactly known, in the structure of the plasma membranes which allows the small, symmetrical urea molecules to penetrate the membrane more easily than the somewhat greater, asymmetric molecules of methylurea. So far it seems as if Type II is in all other respects very similar to Type I. Of the plant protoplasts hitherto studied, some 12% belong to Type II.

Type III. The *Rhoeo* Type owes its name to *Rhoeo discolor*, the epidermal cells of which were found by de Vries to constitute excellent material for plasmolytic permeability determinations. Bärlund (8) observed that these cells are relatively less permeable to all amides than are cells of the Normal Type. Thus, *Rhoeo* cells are less permeable to malonamide than to erythritol, less permeable to urea, thiourea, and methylurea than to glycerol, less permeable to formamide and acetamide than to ethylene glycol, while in all these respects cells of Type I behave in the contrary way. The *Rhoeo* Type could thus also be called the Amidophobic Type. Its peculiarities may easily be explained by assuming that the plasma membranes of this type contain lipoids which are less acidic and thus poorer solvents for the slightly basic amides than are the plasma membrane lipids of the Normal Type. There are not many representatives of this type as extreme as *Rhoeo* itself. Cases more or less intermediate between this type and Type I are, however, encountered.

Type IV (*Beggiatoa* Type). The permeability of *Beggiatoa mirabilis*, extensively studied by Ruhland and his co-workers differs strikingly from the Normal Type: lipid solubility seems in this case to have only a minor influence on the permeation power, while molecular size is the deciding factor. Moreover, the cells of *Beggiatoa* are extremely permeable to all solutes. An organism with permeability properties that resemble those of *Beggiatoa* is the blue-green alga *Oscillatoria* studied by Elo (52). It is not, however, as extreme in its behavior as *Beggiatoa*. Probably these two organisms are also taxonomically related, for *Beggiatoa*, which used to be classified amongst the bacteria, is now often considered to be a colorless member of the Oscillatoriales.

Type V (Diatom Type). Diatoms deplasmolyze with unusual rapidity in hypertonic solutions of several very poorly lipid-soluble substances. Their behavior thus resembles that of *Beggiatoa* and *Oscillatoria*. As may be seen from Table IV there is, nevertheless, a distinct difference between these two types. For in *Oscillatoria* the most striking feature is that even highly lipid-soluble compounds (e.g., propionamide) do not permeate very much faster than, say, glycerol, while the most outstanding property of the diatoms seems to be a considerable permeability to substances of extremely low lipoid solubility and of fairly large molecular size (erythritol, and even sucrose). Under heading IIIC it was mentioned that Bogen and Follman (19) maintain that the rapid deplasmolysis of diatom cells in hypertonic solutions is not due to an unusually great permeability but to active uptake of water and solutes. These authors, therefore, deny the very existence of a special Diatom Type of permeability.

The five permeability types outlined above are by no means sharply separated from each other. At least between Type I, on the one hand, and Types II and III, on the other, there is a quite continuous gradation. Types IV and V seem more isolated, however, according to our present state of knowledge.

It should also be noted that the permeability types discussed do not characterize certain plant species as such: different cells, say, epidermal and subepidermal cells, of one and the same plant may belong to distinctly different permeability types, and even the same cell may at different stages of its development represent different

TABLE IV

PERMEABILITY[a] OF TWO DIATOMS, *Licmophora oedipus* AND *Melosira* SP., AS COMPARED WITH THAT OF *Chara ceratophylla* AND *Elodea* (*Anacharis*) *densa* AS REPRESENTATIVES OF THE NORMAL TYPE AND WITH *Oscillatoria princeps*

Substance	*Elodea*[b]	*Chara*[c]	*Licmophora*[d]	*Melosira*[b]	*Oscillatoria*[d]
Propionamide	115	180	110	84	4.1
Acetamide	71	72	18	14	5.8
Ethylene glycol	51	58	7.2	11	5.4
Methylurea	12	9.2	2.6	3.4	3.2
Urea	4.8	5.4	1.1	1.2	1.0
Glycerol	1.0	1.0	1.0	1.0	1.0
Malonamide	0.61	0.19	1.0	0.5	0.2
Erythritol	0.11	0.06	0.8	0.4	0.04
Sucrose	0.02	—	0.5	0.2	—

[a] Permeability to glycerol put equal to unity.
[b] According to Marklund (112).
[c] According to Collander and Bärlund (35).
[d] According to Elo (52).

permeability types (112). Moreover, Hofmeister (87) found that in certain seasons of the year the subepidermal cells of *Ranunculus repens* behave as Type I, in other seasons as Type III. Finally, a simple rise in temperature increases the permeability toward different substances to very different extents and may thus cause an, at least apparent, transition from Type II to Type I.

In spite of all this, the recognition of separate permeability types seems decidedly useful, for it facilitates survey of the varying permeability properties of different cells. To the present writer it thus seems that Bogen (17) goes too far when he more or less denies the reality of the permeability types, attributing them to differences in active transport, etc.

Figure 2 presents a general view of the permeability differences be-

tween different kinds of plant protoplasts. In this graph each cell type is represented by a vertical line on which the permeation constants (expressed as centimeters per hour) of seven substances are denoted by points on a logarithmic scale. The cell types are arranged according to increasing permeability to erythritol. The sixteen cell types tested

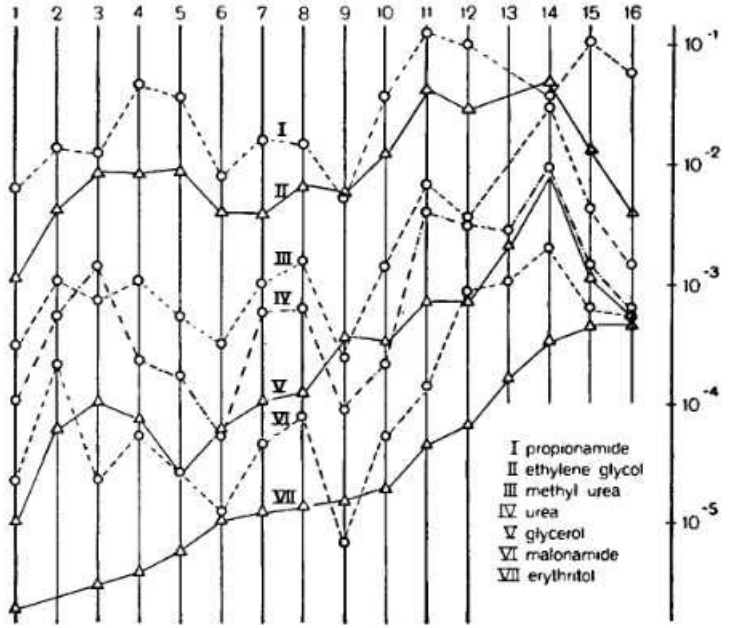

FIG. 2. Permeability of 16 different kinds of plant cells to some nonelectrolytes: *1*, leaf cells of *Plagiothecium denticulatum*; *2*, *Oedogonium* sp.; *3*, root cells of *Lemna minor*; *4*, *Pylaiella litoralis*; *5*, *Zygnema cyanosporum*; *6*, subepidermal cells of *Curcuma rubricaulis*; *7*, *Spirogyra* sp.; *8*, leaf cells of *Elodea* (*Anacharis*) *densa*; *9*, epidermal cells of *Rhoeo discolor*; *10*, epidermal cells of *Taraxacum pectinatiforme*; *11*, internodal cells of *Chara ceratophylla*; *12*, internodal cells of *Ceramium diaphanum*; *13*, *Escherichia paracoli*; *14*, *Oscillatoria princeps*; *15*, *Melosira* sp.; *16*, *Licmophora* sp. From Collander (29).

were intentionally chosen to represent types of plant cells as different as possible. Thus, such very different taxonomic groups as flowering plants, mosses, green algae, charophyta, zygophyta, diatoms, brown algae, red algae, blue-green algae, and bacteria are represented. Also the physiological character of the cells examined varies greatly. Therefore, this graph shows the range of variation in permeability which is encountered in plant cells in general. Of these sixteen cell types, numbers 4, 5, 7, 8, 11, 12, and 13 are seen to represent the Normal

Type; numbers 6 and 10 are intermediate between the Normal Type and the Amidophobic Type; numbers 1 and 2 represent the Normal Type except for a somewhat unusually high degree of amidophilia (malonamide has here a higher permeation rate than glycerol); number 3 belongs to the *Gentiana-Sturmiana* Type; number 9, to the *Rhoeo* Type; numbers 15 and 16, to the Diatom Type.

A general conclusion may be drawn from a collation of data like that given in Fig. 2. Although different plant cells do vary in their permeability properties, this variation cannot obscure the fundamental fact, already stressed by Overton, that there is nevertheless a very striking similarity between the permeability of all protoplasts, especially if we disregard a few extreme cases (*Beggiatoa*, *Oscillatoria*, diatoms). This conclusion would probably emerge even more clearly if the compilation were to comprise a wider range of permeators. Now it comprises only substances of low or moderate permeation power, since the plasmolytic method used in most of these determinations is not suited to give quantitative results with very rapidly permeating substances.

E. SOME ESPECIALLY IMPORTANT NONELECTROLYTES

1. Water

Water has sometimes been supposed to be the most hydrophilic of all substances. In reality, however, there are numerous substances (e.g., urea, polyhydric alcohols, sugars, amino acids, mineral salts) with a lipid:water distribution coefficient which is much smaller than that of water and which must therefore be considered to be more strongly hydrophilic even than water. Nevertheless water is, of course, strongly hydrophilic, and so it may seem surprising that, in spite of this, its permeation power is so great. This, and also the fundamental physiological role of water, makes it understandable that the permeability to water of both plant and animal cells has engaged the attention of such a large number of investigators. It is only a pity that by no means all of them have realized the great difficulties, both technical and theoretical, encountered in such work.

The chief technical difficulties arise from the extreme smallness of the diffusion resistance of protoplasm to water. The permeability to water is usually determined by watching the rate of plasmolysis or deplasmolysis. It may easily happen that the factor that controls the plasmolysis or deplasmolysis rate is not the water permeability of the protoplast, but rather the rapidity with which the plasmolyzing substance diffuses up to the outer surface of the protoplast or from this

surface to the bulk of the bathing fluid. Particularly if the experiments are carried out, not with isolated cells, but with tissue sections, this source of error may be a very serious one. If we wish to measure the water permeability of the protoplast alone, the surrounding cell wall should, therefore, be as permeable as possible, not only to water, but also to the plasmolyzing substance. It is still better, however, to use isolated protoplasts so that the retarding influence of the cell wall is entirely eliminated. But even in such a case the assumption is not fully warranted that the rate of the osmotically induced contraction or expansion of the protoplast depends solely on the water permeability of the protoplast or the plasmatic membranes. For when water is withdrawn from, or taken up by, the protoplast, a layer of higher or lower concentration will be established just inside the protoplast, and this layer of different concentration will counteract the process under investigation. In view of these sources of error, which all tend to depress the water permeability values experimentally obtained below the correct value, it seems probable that most of the values recorded in the literature are actually too low, in some cases perhaps much too low.

Moreover, a quantitative comparison of water permeability and solute permeability is not easy. As stated before, the permeation of a solute across the plasma membrane may be said to be determined by the difference in concentration of that solute on the two sides of the membrane. But this does not hold true as regards the permeation of water. Imagine a 10% solution of, say, sodium chloride on one side of a semipermeable membrane and a 10% solution of sucrose on the other. These solutions contain the same concentration of water, and yet the diffusion pressure of water is nowhere near the same on the two sides of the membrane. Alternatively the permeation of water may be considered as due to differences in osmotic or hydrostatic pressure. A consequence of this has been that the water permeability has generally been expressed in length $\times$ time^{-1} $\times$ pressure^{-1} units. Such a procedure is in itself correct, but it has the regrettable drawback that the water permeability values expressed in this way are not comparable with other permeability values expressed in length $\times$ time^{-1} units.

Attempts have therefore been made to calculate permeation constants for water in virtually the same manner as for solutes. These attempts have for the most part been based on the assumption that a concentration gradient of solute determines a concentration gradient of water of equal magnitude but opposite sign and that under these conditions the diffusion of water molecules quantitatively obeys the same laws as does that of the solute molecules in a dilute solution. According to Jacobs (99) neither of these assumptions appears to be warranted. On the other hand, Bochsler (14) found that the permea-

tion rate of water is proportional to the difference between the actual water concentration of the cell sap and its water concentration after osmotic equilibrium has been established.

There is, however, a simple method of determining the water permeability in exactly the same way as the permeability to solutes, namely to watch the permeation of isotopic water, either deuterium oxide or tritium oxide, which, when mixed with ordinary water, should behave essentially like any other solute. The theoretical drawback to this method, i.e., that heavy water permeates slightly more slowly than ordinary water, seems to be of no great significance.

TABLE V
PERMEABILITY OF SOME PLANT PROTOPLASTS TO WATER[a]

Cell type	Method	P_f	P_d	Authority for measurement
Fucus vesiculosus, egg cell	Osmosis	0.0008–0.001	—	(138b)
Allium cepa, parenchyma cell	Osmosis	0.002	—	(110b)
Salvinia natans, cell of water leaf	Plasmolysis	0.003	0.004[b]	(90b)
Nitella flexilis, internodal cell	Osmosis	0.04–0.11	—	(102)
N. mucronata, internodal cell	Isotope	—	9.0	(32)
Nitellopsis obtusulus, internodal cell	Osmosis	0.006	0.43[c]	(127)
N. obtusulus, internodal cell	Isotope	—	1.0	(171)
Vallisneria spiralis, epidermal cell	Plasmolysis	—	0.004[b]	(90b)
V. spiralis, mesophyll cell	Plasmolysis	—	0.016[b]	(90a)
Spirogyra sp.	Plasmolysis	—	0.011	(14)

[a] Permeability is expressed either in centimeters per hour per atmosphere (P_f) or in centimeters per hour (P_d). Temperature about 20°C.

[b] Value has been calculated by Bochsler (14).

[c] Value has been calculated by Wartiovaara (171).

According to experiments carried out with the isotope method (32), water permeates through the protoplasts of *Nitella* about 20,000 times more rapidly than urea and some 500,000 times more rapidly than glycerol. Thus water has, in fact, an extremely great permeation power in comparison with most other substances.

But the question of water permeability has also another aspect, for, as seen from Table V, the flow rate of water through a plant protoplast is usually even less than 0.1 mm per hour under the influence of a pressure difference of 1 atm. From this point of view, then, the actual resistance of protoplasm to the flow of water may be said to be very great. This statement seemingly contradicts that given previously, according to which water has an exceptionally great permeation power. This apparent contradiction is, however, readily resolved when we realize that the diffusion resistance of living protoplasts toward all

substances is, in fact, very great. Although their permeability to water is much greater than their permeability to almost all other substances, their *absolute* permeability even to water is nevertheless rather low.

This is a fact which must not be forgotten when the course of the transpiration stream through parenchymatous tissues, e.g., in root and leaves, is being discussed. In streaming through such tissues, the water evidently has to choose between two alternative pathways, namely, (a) across the protoplasts, and (b) through the cell walls flanking the protoplasts. In reality both paths will, of course, always be used simultaneously, and it seems *a priori* clear that the intensity of each partial stream will be inversely proportional to the resistances encountered along each path. (Cf. Ohm's well-known law which governs the relative intensities of electric currents flowing in parallel.) Regrettably, however, the magnitudes of these resistances are not yet sufficiently known, and hence it is not yet possible to calculate the relative intensities of the two partial streams.

2. *Oxygen*

Free oxygen is one of the most rapidly penetrating substances. Its extremely rapid penetration through the protoplasm can be inferred from a simple observation made in connection with Engelmann's well-known bacterial method of demonstrating the evolution of oxygen in photosynthesizing cells. To this end a filament of, say, *Spirogyra* sp. is mounted in water containing positively aerotactic bacteria and covered with a glass slip whose edges are sealed to prevent the entrance of air. Now, if the preparation is exposed to light so that photosynthesis is started in the *Spirogyra* cells, the bacteria will not accumulate evenly around the photosynthesizing cells but almost exclusively just outside the individual chloroplasts. This indicates that the oxygen produced by the chloroplasts diffuses straight out from the cells without being so much retarded by the plasma membrane as to have time to spread to the sides before leaving the cells (Veijo Wartiovaara, private communication). There are also a few quantitative evaluations of the permeability of algal and bacterial cells to oxygen (137).

The great permeation power of oxygen is understandable in view of its considerable lipid solubility and the smallness of its molecules. Besides, all other gases, too, must have a very great permeation power, for they are all very lipid-soluble and/or have very small molecules.

3. *Sugars and Amino Acids*

These are among the most lipid-insoluble substances known. It is therefore only natural that plant protoplasts, when, for instance, plas-

molytically studied should prove more or less impermeable to them. This applies even to such a relatively small-molecular substance as glycine. On the other hand, a continuous consumption of sugars and amino acids must be assumed in growing and respiring cells. But, as already pointed out in IIIC, the uptake of these substances seems largely to be due to processes of active transport.

VIII. Permeability to Salts and Ions

For several reasons it is much more difficult to study the permeation power of salts and ions than to measure that of nonelectrolytes.

Owing to the great electrical charges of the ions, measurable amounts of any single ionic species cannot be taken up or given off by a cell unless the ions in question are either accompanied by an equivalent amount of oppositely charged ions or exchanged for an equivalent amount of the same electrical sign.

Another very great difficulty is that the true permeability to ions, at least of most plant protoplasts, seem to be very small and that the transfer of ions through the plasma membranes is principally due, not to simple permeation, but to poorly known metabolic forces combined with electrical gradients of unknown sign and magnitude. While nonelectrolytes mostly tend to distribute themselves between the cell sap and the surrounding medium in such a manner that their final concentrations are approximately the same in each solution, such a distribution almost never occurs in the case of ions (see this volume, Chapter 4). A discussion of salt uptake by plant cells in terms of simple permeation would therefore be not only difficult but misleading.

Therefore, and in spite of a vast literature in this field, our present knowledge of the permeability of plant protoplasts to salts and ions is still far from being as exactly established as our knowledge concerning the permeability to nonelectrolytes. In fact, although permeability constants to express quantitatively the permeability of plant protoplasts toward numerous nonelectrolytes have been calculated, no permeation constants for salts and only extremely few for ions are so far available.

The active transport of ions will be thoroughly treated in this volume, Chapter 4; here only the true permeability to salts and ions will concern us.

The first observations concerning the salt permeability of plant cells were all based on plasmolytic experiments. Many years ago de Vries (168) and Overton (121) found that concentrated solutions of such salts as sodium chloride or potassium nitrate produce a permanent plasmolysis. From this they concluded that the protoplasts are, in general, more or less impermeable to such salts. Later investigators, using a

more refined plasmolytic technique have achieved somewhat more exact results. Thus Fitting (58), in his experiments with epidermal cells of *Rhoeo discolor*, was able quantitatively to measure the slow deplasmolysis of the cells in solutions of several alkali salts. Presuming that the rate of deplasmolysis is a measure of the penetration rate of the salt, he assumed that the penetration rate decreases in the order $K > Na > Li$ as far as cations are concerned, while, in the case of the anions, sulfates penetrate considerably more slowly than chlorides, bromides, chlorates, and nitrates. In solutions of alkaline earth salts (chlorides and nitrates of Mg, Ca, Sr, and Ba) no deplasmolysis could be detected. The most rapidly penetrating salts seemed to have an initial permeation rate slightly less than that of glycerol; however, in a few hours the deplasmolysis rate decreased considerably, indicating, according to Fitting, a strong reduction in permeability to the salts in question. Similar results obtained with other cells were reported later by several other investigators. However, the exact interpretation of such seemingly simple experimental results is far from clear. Thus it is almost impossible to decide to what extent the results may have been influenced by factors such as a possible exosmosis of normal components of the cell sap or by active ion transport. Moreover, if an exchange of ions between the protoplast and the medium occurred, it would not, of course, be detectable by plasmolytic methods.

Evidently, then, the plasmolytic experiments must be supplemented, especially where ion permeability is concerned, with studies based either on chemical analyses or on the use of radioactive isotopes. A combination of chemical and radioactivity determinations is still better.

For a long time it has been known that the sap of some *Valonia* species contains high concentrations of potassium ions but only relatively low concentrations of sodium ions, while in the sea water from which the algae must have taken up all their constituents, the ratio between potassium and sodium is just the opposite. This is, in fact, a situation which is typical of a multitude of both plant and animal cells: with but few exceptions potassium dominates in the interior of the cells in spite of the frequent dominance of sodium in the fluids bathing the cells. Thus one or both of these ionic species is actively transported across the plasma membranes. At the same time, however, these membranes must be poorly permeable to the ions in question, for the greater the leakage occurring across them, the greater is, of course, the work required to maintain the constant ionic difference between the extra- and intracellular fluids.

With cells of *Nitella* and some other characeans, somewhat more detailed experiments have been carried out. Thus, for instance, some

thirty years ago Hoagland *et al.* (72) found that when *Nitella* cells are placed in a dilute bromide solution a very slow exchange of bromide ions for chlorine ions occurs, so that within about 40 days a kind of steady state is attained. However, as pointed out by Steward and Millar (156), in these experiments a large population of *Nitella* cells was used, and so the probability exists that the bromide was actively taken up preferentially by the younger, still growing cells, while the chloride issued principally from more senescent ones. The exchange of bromide ions for chloride ions may thus in reality have occurred much more slowly than one would at first sight have been inclined to suppose. In other experiments (30), characean cells rich in potassium ions have been kept in solutions of rubidium salts. The exchange of rubidium for potassium was then found to occur at least a million times as slowly as an exchange of these cations by free diffusion through a water layer of the same thickness as the protoplasmic layer, or roughly a thousand million times as slowly as by diffusion through a water layer of the thickness of the plasma membrane. It thus seems fairly well established that cells of this type are almost impermeable to cations as well as to anions. In Section IV,B,2 it was stated that inanimate membranes may easily be prepared which are permeable to either cations or anions alone. Several animal protoplasts also seem to be more readily permeable either to positive or to negative ions. It may therefore be pointed out that among plants no clear examples of either cation- or anion-permeable protoplasts are so far known.

Besides, measurements of the electrical conductivity of living cells also show that their membranes are almost impermeable toward ions. Thus, for instance, the direct current resistance of *Nitella* cells has been found to be of the order of magnitude of 100,000 ohm per square centimeters. [Cf. Cole (28), Danielli (42), Blinks (13), and Umrath (164).]

More quantitative measurements of the permeability to ions have been carried out by Holm-Jensen *et al.* (89). Cells of some of the Characeae had been kept for several weeks in a constant milieu, and the authors therefore assumed that a steady state had been reached in which the loss of any ions by diffusion was balanced by active uptake of the same ion. When now one of the ions present in the medium is labeled by the addition of an infinitesimal amount of a radioactive isotope, without any appreciable alteration of the concentration of the medium, the protoplasm and the sap of the cells will gradually become more radioactive and the rate of increase of their activity can be used to estimate the rate of diffusion of the cation in question through the protoplasm. In this way the permeability of the protoplasts to potassium

ions was found to be roughly 1×10^{-8} cm per second for *Nitellopsis* (= *Tolypellopsis*) and about 2×10^{-9} cm per second for *Nitella*, while the corresponding values for sodium ions were about 4×10^{-9} cm per second in the case of *Nitellopsis* and 0.4×10^{-9} cm per second in the case of *Nitella*. This means that the half-exchange times would be something like 20–400 days for these ions and cells. At any rate it is clear that these ion exchanges proceed exceedingly slowly, approximately as slowly as the permeation of sugars, for instance. On the other hand, Brooks (94) found a considerably more rapid uptake of radioactive potassium and sodium ions into the sap and especially into the protoplasm of *Nitella* cells. A critical repetition of these experiments therefore seems desirable.

Among experiments concerning the ion permeability of yeast cells, those of Conway and his associates [cf. Conway (39)] may be cited. They found that if resting yeast cells are shaken anaerobically in a solution containing labeled potassium ions, after some hours only a small percentage of the potassium ions from the external solution have mixed with the internal potassium ions. If, on the other hand, the yeast is shaken in the same solution in the presence of air, labeled potassium ions will enter more readily so that, after 1 hour, the mixing may have proceeded to some 40 to 50%, but this entry is almost entirely inhibited by cyanide. Especially in view of the small dimensions of the yeast cells their permeability toward potassium ions must thus be designated as very low. A high degree of impermeability of yeast cells to anions has likewise been reported (59).

There also exist a great number of studies on the ion exchange between excised roots or disks of various storage tissues, on the one hand, and aqueous salt solutions, on the other, but the proper interpretation of their results is no easy matter (cf. 54, 55, 107, 140, 158). In almost all these investigations there has been found a conspicuous dualism in regard to ion uptake: after an initially rapid uptake, or exchange, of ions, there follows a period of very much slower uptake or exchange. The first of these processes is largely independent of temperature, oxygen supply, and metabolic inhibitors, while the second step is strongly affected by all these factors. Hence it seems natural to assume that the uptake during the initial phase is essentially of the "passive" type, while the second one is metabolic in nature (cf. Chapter 4 in this volume). So far virtual agreement exists between most investigators in this field. But, as stated in Section VIA, the parts of a tissue to which strong electrolytes have free access during the first phase is still largely an open question: Is it to cell walls and intercellular spaces only, or to the cytoplasm or to parts of it, also? The answer to

this question will prove of fundamental importance to the understanding of the influence of ions upon plant protoplasts.

Irrespective of whether the main diffusion resistance toward ions is located in both outer and inner plasma membranes or in the inner alone, it seems that almost all plant protoplasts possess a great, or even a very great, diffusion resistance toward ions. Thus, it is evident that active transport rather than diffusion is primarily involved in the entry of ions into cells (see Chapter 4 of this volume).

There may, however, exist a few exceptions to this general rule.

One such exception, and indeed a very outstanding one, is that displayed by the chief example of the ultrafiltration theory, *Beggiatoa mirabilis*, which seems to possess an extremely high permeability to salts (146, 147). But surely it is no mere chance that the cells of this organism are almost devoid of turgor: a certain degree of semipermeability is obviously indispensable for the development of turgor pressure.

Certain brown algae living in the tidal zone where they are exposed daily to great concentration changes are also characterized by an exceptionally high salt permeability (12), and the same seems to hold true for numerous diatoms (81). Perhaps the high salt permeability of these organisms is to be interpreted as an adaptation which makes it possible for them to endure the concentration changes in their natural habitats.

Concerning the ion permeability of bacterial cells there still exists some diversity of opinion. Thus, according to Roberts *et al.* (139) the cells of *Escherichia coli* are freely permeable to most, if not all, ions, so that equality of concentration with the ambient solution is reached within 5 minutes. On the other hand, several other investigations [Fischer (57); Hill (69); Collander (33); Mitchell and Moyle (112a)] clearly show the existence, in a variety of bacterial cells, of a peripheral osmotic barrier more or less impermeable to ions. It therefore seems probable that the relatively rapid uptake and release of solutes often observed in bacterial cells may depend in part on their exceptionally great surface:volume ratio and in part on more or less specific ion exchange mechanisms.

IX. Permeability to Acids and Bases

A. Introduction

The study of permeability to acids and bases is beset with special difficulties. First of all, it must be remembered that their hydrogen and hydroxyl ions, if present in too high concentrations, will display toxic

effects. In all permeability experiments with acids or bases, it will therefore be especially important to ascertain whether the cells experimented with are still in a normal state at the end of the experiment. Otherwise there is the possibility that the observed penetration of acids or bases may be only a consequence of the protoplasts having lost their normal state of restricted permeability.

Another complication is that acids and bases may penetrate the cells as either (a) undissociated molecules, (b) ions, or (c) molecules and ions at the same time. In the preceding section we have seen that plant protoplasts are, in most cases at least, relatively impermeable to ions. We have thus to expect that weak or moderately strong acids and bases will penetrate exclusively, or at least preferentially, as undissociated molecules. Wide experience with numerous acids and bases shows that this is in reality so (151). This fact must, of course, be taken into account in calculating the permeability constants: the effective concentration gradient is not that of the total acid or base but that of the undissociated molecules alone. Evidently, then, the final equilibrium attained will be a function of the extra- and intracellular hydrogen ion concentrations, on the one hand, and of the dissociation constant of the penetrating acid or base, on the other (103). Thus, to take a specific example, ammonia molecules entering the acid sap of a cell will instantaneously be converted into ammonium salts of the acids present in the sap. These ammonium salts (i.e., the ammonium ions) are unable to penetrate the protoplast, while the ammonia molecules ($NH_3 + NH_4OH$) penetrate it with extreme rapidity. The consequence of this will be that the ammonia is very effectively trapped, or accumulated, in the cell sap. This is not, of course, an example of the active transport capacity of the cells but only a case of diffusive or "metaosmotic" solute uptake in the sense of Bogen (15). By watching the rate of accumulation of ammonia it is therefore virtually possible to calculate its permeation constant provided the concentrations of the undissociated ammonia molecules outside and inside the cell are known.

B. Acids

The cell sap often contains lipid-insoluble acids such as oxalic, malic, tartaric, and citric acids in remarkably high concentrations. On the other hand, acetic, lactic, and pyruvic acids, although frequently arising in cell metabolism, are rarely found to accumulate in cells as free acids. It seems plausible to assume that this striking difference in the occurrence of the two groups of acids is due to differences relating to their permeation power: the acids of the second group possess a considerable lipid-solubility and have such a great permeation power that

they simply cannot be kept accumulated in any cells whereas, on the other hand, the plasma membranes are practically impermeable to the acids of the first-mentioned group, which, therefore, if once accumulated in the cell sap, will remain there for unlimited periods of time.

One way of investigating the entrance of acids into plant cells is to observe the color changes brought about in cells which contain anthocyanins in their sap. Unfortunately, however, the anthocyanins of most cells are relatively insensible to slight increases in acidity (23, 49). Nevertheless, as was found by Jacobs (93), the pigment of the petal cells of *Symphytum peregrinum* is so sensitive to even a slight increase in the acidity of the sap that the entrance even of such an extremely weak acid as carbonic acid is clearly visible. With these cells Jacobs showed that carbonic acid or its anhydride CO_2 has a unique permeation power. Thus the cells "may be caused to develop an intracellular acidity in a solution of CO_2 in $M/2$ $NaHCO_3$, which has the alkaline reaction of pH 7.4, practically as rapidly as in a solution of CO_2 in distilled water, of pH 3.8; i.e., with an apparent hydrogen ion concentration 4,000 times as great, while in distilled water of pH 5.5–6.0 there is no change, although the apparent H-ion concentration is perhaps 100 times as great as in the first-mentioned solution." These seemingly paradoxical results are easily explained if the H-ions do not penetrate appreciably, but only the CO_2 (and H_2CO_3) molecules do so. Hydrogen sulfide is another weak acid which behaves toward living cells very much like carbonic acid (119).

An ingenious method of determining quantitatively the permeation power of both weak acids and weak bases was invented by Jacobs (97). Aqueous solutions of, say, ammonium acetate are always partially hydrolyzed into acetic acid and ammonia:

$$CH_3CONH_4 + H_2O \rightleftharpoons CH_3COOH + NH_4OH$$

Now, if we plasmolyze plant cells with an ammonium acetate solution, the salt as such will penetrate the protoplasts only very slowly. On the other hand, both acetic acid and ammonia molecules will enter very rapidly and will combine in the cell sap so that ammonium acetate is again produced. As a consequence of this, deplasmolysis will occur and its rate will depend primarily on the entrance rates of acetic acid and ammonia. This method has two great advantages. First, acid and base occur in such low concentrations that toxic effects are largely avoided. Secondly, although the acid and the base, if studied separately, would penetrate the cells so rapidly that exact measurements of their penetration rates would be extremely difficult, their joint penetration from the salt solution may nevertheless be readily measured owing to the very

low concentration of the permeating molecules. From experiments carried out with this method it can be deduced that the permeation powers of the fatty acids increase in the order acetic < propionic < butyric < valeric acid. The influence of lipid solubility seems thus in this case more important than that of molecular size. The first member of this homologous series, formic acid, seems however to be exceptional.

Indoleacetic acid and other growth regulators of analogous composition constitute a physiologically important group of weak acids. Their physiological activity is, at least approximately, proportional to the concentration of the undissociated acid molecules in the external solution (120). The lipid solubility of all these substances is so great that they will readily penetrate all sorts of plant protoplasts. Also they should be accumulated from acid solutions in the more neutral or alkaline regions of the plant, such as, the cytoplasm and the content of the sieve tubes. According to Johnson and Bonner (100) phenoxyacetic acid, however, may be taken up, or bound, in several different ways.

C. BASES

Turning now to the permeation of bases, we shall find among them a state of affairs similar to that among the acids. Thus the extremely high permeability of all living protoplasts to carbonic acid and/or its anhydride has its counterpart in their behavior toward ammonia ($NH_3 + NH_4OH$). Just as living cells may turn more acid in an alkaline bicarbonate solution, so their pH may change in an alkaline direction when they are placed in a slightly acid solution of, say, ammonium chloride, owing to the very rapid penetration of the ammonium liberated by hydrolysis of the ammonium salt (92).

There are numerous other weak bases, such as alkylamines, alkanolamines, and the natural alkaloids, which behave in much the same manner toward living protoplasts as does ammonia (65, 122). A more quantitative comparison of the permeation powers of several such bases has been carried out by Äyräpää (6). His experiments were performed with bakers' yeast. The method was based on the color change caused by the penetration of the bases into yeast cells stained with neutral red. The time required to reach a certain standard color was used as an inverse measure of the penetration rate. His results indicate, as is seen from Fig. 3, that lipid solubility is a most important factor here. Thus even such very large alkaloid molecules as those of atropine (mol. wt., 289), cocaine (mol. wt., 303) and thebaine (mol. wt., 313) were found to penetrate the cells very rapidly. On the other hand, the very smallest molecules (ammonia, methylamine, hydrazine) showed permeation powers about 100–1000 times as great as those of somewhat

larger molecules of corresponding lipid solubility. [Kral (105), using *Tradescantia* cells as his test object, has recently, with another technique, obtained results which are not quite in accord with those of Äyräpää.]

Like the strong acids (HCl, HNO_4, H_2SO_4, etc.), the strong bases

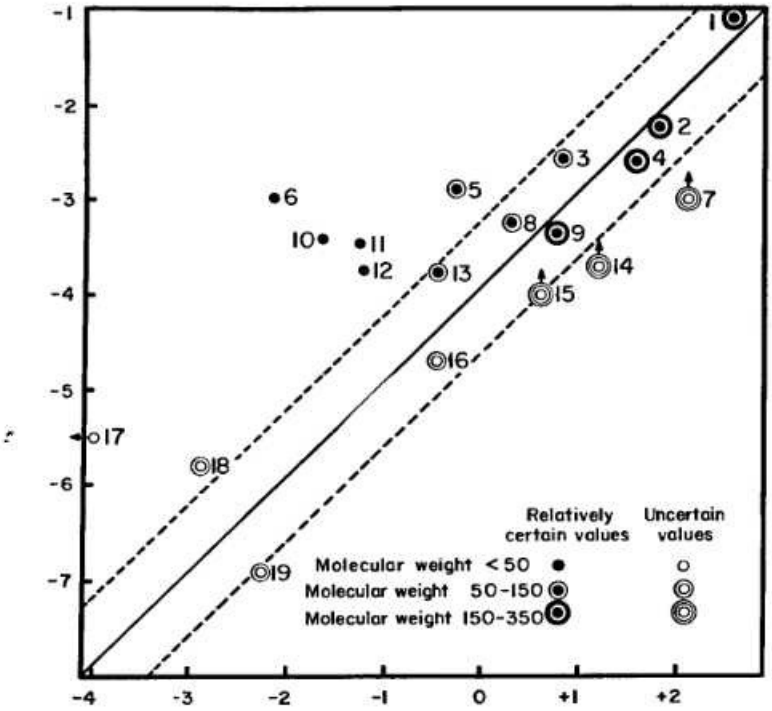

FIG. 3. Correlation between the permeation power of several bases toward yeast cells, on the one hand, and their relative ether solubility and molecular weight, on the other. Abscissa: log distribution coefficient ether : water; ordinate: log $PM^{1/2}$. The points are: *1*, diisoamylamine; *2*, sparteine; *3*, isoamylamine; *4*, novocaine; *5*, diethylamine; *6*, ammonia; *7*, cocaine; *8*, triethylamine; *9*, ephedrine; *10*, methylamine; *11*, dimethylamine; *12*, ethylamine; *13*, trimethylamine; *14*, thebaine; *15*, atropine; *16*, diethylethanolamine; *17*, hydrazine; *18*, ethanolamine; *19*, diethanolamine. From Äyräpää (6).

(NaOH, KOH, $Ca(OH)_2$, tetraalkyl ammonium bases, etc.) have almost no permeation power toward normal, uninjured protoplasts (23, 65).

X. Permeability to Dyes

Theoretically the permeability to dyes should already have been treated in the discussion devoted to the permeation of strong and weak electrolytes. However, the vital stains form such a peculiar group of

permeators and have given rise to such a vast literature that their separate consideration seems appropriate.

It has already been pointed out that, although investigations into the permeability of living cells to dyes may at first sight seem very promising, they are in reality beset with numerous pitfalls the danger of which should not be underestimated [cf. Drawert (50)].

Thus most of the commercially obtainable dyestuffs contain large amounts of impurities. Many dyestuffs are in fact mixtures of several dyes differing from each other not only in chemical constitution but also in permeation power. A notable example of this is the much-used vital stain methylene blue. Pure methylene blue is tetramethylthionine, which is a strong base. It is in itself a poor vital stain. In aqueous solutions, however, especially at pH values above 9.0, it is gradually oxidized to trimethylthionine, which is a much weaker base and has a much greater permeation power. So it comes about that most vital staining with "methylene blue" is in fact due to impurities in the dyestuff used. The Biological Stain Commission (United States) has done much to facilitate the supply of stains of known chemical and physiological properties. (Cf. 38.)

Another drawback of many dyes, and especially of almost all basic dyes, is their great toxicity to living cells. In order to avoid damage to the cells, such dyes must therefore be used in very low concentrations. In layers less than, say, 1 mm thick such dilute stain solutions appear colorless. Obviously, then, the entrance of such dyes into cells of microscopic dimensions cannot be observed unless the dye is efficiently accumulated in the cell. This fact has given rise to endless discussions as to whether the nonappearance of a stain in cells is due to failure of the dye to penetrate the protoplasts or only to failure of the cells to bind the dye. There is, of course, no single answer to this question: each case requires special study.

Fluorescent dyes are often visible even if present in extremely low concentrations. In recent years therefore studies of their uptake have aroused much interest.

Exact measurements of the dye concentrations attained in cells of microscopic dimensions have hitherto proved very difficult. Only quite recently have instruments suitable for such measurements been devised (10). The possible intracellular reduction of dyes to colorless compounds, the strong adsorption of dyes by many cell walls, and the colloidal or semicolloidal nature of many dyes are other difficulties encountered in studies of the permeability of protoplasts to dyes.

It is not surprising, therefore, that the numerous attempts to test the permeability hypotheses by vital staining experiments have, on the

whole, yielded rather disappointing results. "They have not only not as yet furnished a solution of the general problem of cell permeability but they have, if anything, been responsible for even greater confusion than existed before." These pessimistic words of a distinguished reviewer of this field (93) some thirty years ago are still difficult to refute.

Before discussing the details concerning the permeation of dyes, it should be pointed out that the permeation power and other physiological properties of dyes depend, to a very large extent, on their ionization, and the dyes may thus be divided into two main classes, i.e., basic and acid dyes.

When a dye is classified as "basic" this means that it is a salt of a colored base with some colorless acid, such as, for instance, hydrochloric or sulfuric acid. Most of the dye bases are weak bases with an ionization constant between about 10^{-3} and 10^{-8}. There are also, however, a few dye bases which may be called strong. The already mentioned tetramethylthionine or methylene blue base is an example of this.

Acid dyes, on the other hand, are salts of colored acids with colorless bases such as, for instance, sodium or potassium hydroxide. Among the dye acids some are weak acids which owe their acidic character to the presence of carboxyl or phenolic hydroxyl groups. The nitrophenols, phthaleins, and fluoresceins may be mentioned as examples of such dyes. On the other hand, numerous acid dyes are obtained by sulfonating basic dyes; they thus contain the —SOOH group and have the character of strong acids.

Numerous basic dyes are effectively accumulated by many, but not all, plant cells. The mechanism of this accumulation was outlined by Pfeffer in his fundamental investigation in the year 1886 (131) on the uptake of synthetic dyes by plant cells. Since then further details of the process have been filled in by McCutcheon and Lucké (111), Irwin (91), Jacobs (97), Kinzel (103), and others. There are two variants of this mechanism. The first of them is virtually identical with the mechanism of accumulation of ammonium ions already described (IX). The nonionized lipid-soluble dye base molecules enter the cells very rapidly. If the cell sap is acid enough, they form salts with the acids of the sap. These salts have practically no permeation power. Once formed, therefore, they cannot leave the cell again but are trapped there. New amounts of the dye base will continue, however, to enter until the concentration of the nonionized dye base is the same in the cell sap as in the surrounding solution. The upper limit of the accumulation brought about in this way is thus a function of the H-ion con-

centrations of the sap and of the ambient fluid, on the one hand, and of the ionization constant of the dye base on the other. [For particulars, see (103).]

Cells trapping basic dyes in this way, i.e., owing to their acid content alone, are said by Höfler and Schindler (84) to have "empty saps." There are, however, other kinds of cell saps too—"full saps" as Höfler called them. They contain other substances capable of binding dye bases. The chemical nature of these substances is known only in a few cases. So far it is not possible to state quantitatively the maximal accumulation capacity of such cells.

From what has been said above, it should be clear that the uptake of basic dyes differs from the more familiar uptake of, say, lipid-soluble nonelectrolytes only in one respect, namely, in that the base molecules do not remain free in the cell sap but are bound in some way. This binding of the dye bases does not prevent the calculation of their normal permeation constants. At least a minimum value for the permeation constant is readily obtainable if, as a first approximation, we assume that the concentration of the nonionized dye base molecules in the sap, during the time period in question, remains equal to zero. So far, however, very few permeation constants calculated in this way are to be found in the literature [e.g., Bartels (10)].

Turning now to the sulfonic acid dyes, we are confronted with substances which behave in quite another manner than the basic dyes. Thus, the sulfonic acid dyes are accumulated in comparatively few kinds of cells, and this accumulation is not due to a trap mechanism but to active transport (51). In most kinds of plant cells no entrance at all of the sulfonic acid dyes can be observed even in hours or days, in spite of the fact that most of these dyes are fairly nontoxic and can thus be used in high concentrations.

Is this nonentry of the sulfonic acid dyes caused by "lack of affinity" for these dyes on the part of the cell content or by true impermeability of the protoplasts toward them? That the latter alternative is the right one is shown, *inter alia*, by the following experiments (36): Cells of *Nitellopsis* were kept 1–2 weeks in fairly concentrated solutions of such dyes. At the end of the immersion period no traces of the dye could be detected in the isolated cell sap. At any rate the dye concentration in the sap was less than 1/1000 of its concentration in the bathing fluid. This cannot have been due to a continuous intracellular decolorization of the dye, because in special experiments with very small amounts of bathing fluid no decrease of its dye concentration could be detected. Nor can the absence of dye in the cell sap be explained by assuming that the dye is insoluble in the sap or that it is negatively adsorbed by some

colloids of the sap, for analyses of the sap showed that it is essentially a not too concentrated aqueous salt solution containing only very low concentrations of organic substances.

The permeation power of the weak dye acids has not been much studied. It seems, however, possible that fluorescein, like the auxins, is accumulated in the sieve tubes. Such an accumulation, if really occurring, could well be the counterpart of the trapping of weak bases in acid saps, for the sieve-tube content is distinguished by such extremely high pH-values as 7.4–8.7 (176).

XI. Influence of External and Internal Factors on Permeability and Active Transport

A. INTRODUCTION

All properties of the living protoplast are subject to changes brought about by a great variety of internal and external factors. It is therefore only natural to assume that their permeability and their capacity for active transport also fluctuate according to existing conditions. The literature contains, in fact, an abundance of hints, assumptions, and statements concerning permeability changes in living protoplasts. Many authors seem, indeed, to regard this changeability as the most significant quality of protoplasmic permeability. It is also a very widespread opinion that these changes in the permeability of the protoplasts are of essential importance to the life processes occurring in the cells: an increase in permeability will, according to these views, promote the course of the life processes, while a decrease in permeability is supposed to slow them down. Experimental verification of these assumptions has not always been thought to be necessary, so that the permeability-change concept has largely served as a kind of magic formula considered adequate to solve even the most complex problems in a simple manner. Moreover, when verification has been attempted, the success achieved has often been about inversely proportional to the exactness of the methods used.

The craze for discovering permeability changes was understandable as long as it was thought that the exchange of substances between cells and their surroundings depended solely, or at least primarily, on the magnitude of cell permeability. But now, since it has been realized that active transport plays a major role in this process, it seems *a priori* more likely that cell activity will be controlled rather by the intensity of active transport than by variations in cell permeability. It also seems that the capacity for active transport is much more susceptible to both external and internal influences than is permeability proper.

B. INFLUENCE OF SINGLE FACTORS

1. Temperature

Temperature is one of those factors whose influence on both permeability and active transport is most indubitable and apparent. Indeed, the very fact that temperature is a measure of the mean kinetic energy of the molecules and ions makes it self-evident that all permeation and transport processes must of necessity be influenced by temperature.

The influence of temperature on chemical, physical and physiological processes is often expressed in terms of Q_{10}. This temperature coefficient, as it is called, is the ratio of the velocity constant of a process at a given temperature to its velocity constant at a temperature 10°C lower. Thus

$$Q_{10} = \frac{k_t}{k_{t-10}}$$

where k_t and k_{t-10} are the velocity constants of the process at the temperatures t and $t - 10$, respectively.

For a long time it has been known that the temperature coefficient of diffusion in aqueous solutions is generally of the magnitude 1.2–1.4, while that of chemical reactions is much higher, i.e., about 2–3. Now, the Q_{10} values of cell permeability are also very high, mostly between 2 and 5, sometimes even still higher. In the past this was frequently regarded as an indication of the "chemical nature" of the permeation process. This conclusion was, however, unwarranted, for we now know that diffusion processes in highly viscous media often show high temperature coefficients. It now seems more plausible to assume that the great influence of temperature on permeation rates might be, in the first place, an indication of a high viscosity of the plasma membranes. But the temperature coefficients of permeation are such complex quantities that a thorough analysis of them presents very great difficulties (173).

As is seen from Table VI, the temperature coefficients of permeation vary considerably, depending on the character of the permeator. Some time ago it was held that the coefficients were smaller in the case of rapidly permeating substances and greater in the case of slow permeators. According to Wartiovaara (173), however, it is more correct to say that the temperature coefficients of small molecules are, on the whole, lower than those of larger ones. Theoretically such a rule would not be surprising, since it seems comprehensible that the activation energy of the permeation process should increase with the bulkiness of the permeating molecules.

If the structure of the plasma membrane changes with the temperature, the temperature coefficient will, of course, be influenced by such changes. However, insofar as it is hitherto known, the value of the temperature coefficient is fairly constant within the whole temperature range so far studied, i.e., between about 0° and 30°C. It thus seems as though at least no sudden major structural changes occurred within these temperature limits.

TABLE VI

TEMPERATURE COEFFICIENTS (Q_{10} VALUES) OF THE PERMEATION OF DIFFERENT NON-ELECTROLYTES WITHIN CERTAIN TEMPERATURE INTERVALS AS COMPARED WITH THE PERMEATION CONSTANTS OF THE SAME SUBSTANCES AT 20°C[a]

Substance	Temperature interval (°C)			P (cm/hour)	Test object
	0–10°	10–20°	20–30°		
Urea	2.5	2.6	2.8	4×10^{-4}	*Chara ceratophylla*
Methanol	2.6			2.0	*Nitella mucronata*
Ethanol	2.7			2.0	*Nitella mucronata*
Propanol	2.7			3.3	*Nitella mucronata*
Ethylene glycol	2.3	2.9	3.5	0.010	*Nitellopsis obtusulus*
Methyl carbamate	3.2	2.9	—	0.75	*Nitellopsis obtusulus*
Glycerol	—	3.5	8	8×10^{-6}	*Nitellopsis obtusulus*
Tetraethylene glycol	—	4.9	—	5×10^{-4}	*Nitellopsis obtusulus*
Trimethyl citrate	—	5.5	—	0.05	*Nitellopsis obtusulus*
2,3-Butylene glycol	7.4	5.9	4.8	0.016	*Nitellopsis obtusulus*

[a] Mainly after Wartiovaara (173).

The temperature coefficient of active transport processes seems also to have values higher than 2.

2. *Light*

The literature contains numerous statements concerning permeability changes, especially permeability increases, caused by visible and invisible radiation [cf. Brauner (22)]. There are, however, many negative statements, and so this question is still somewhat obscure.

On the other hand, there can be no doubt concerning the favorable effect of light on the active uptake of salts by algae and other chlorophyll-containing cells. Probably this effect is, directly or indirectly, connected with photosynthesis.

3. *H^+ and OH^- Ions*

As already stated (Sections IX and X) the uptake of weak acids and bases is strongly influenced by the pH value of the ambient

solution. This is, however, primarily an influence on the ionization of the penetrating substances rather than an influence on the cells themselves. On the other hand, if the proteins of the plasmalemma participate at all in the uptake of ions, the pH of the medium may be expected to affect cell permeability, since it is well known that proteins on the acid side of their isoelectric point will combine with anions, while on the alkaline side cations are adsorbed. Experimental evidence of such effects is, however, meager (55).

4. Other Ions

There are especially in the somewhat older literature, many statements that certain univalent cations such as Na^+ and K^+ tend to increase cell permeability, while Ca^{++} and also some other bi- and trivalent cations have the opposite effect. [Concerning the vast literature on "ion antagonism," see the textbooks of Höber (76) and Heilbrunn (68).] A more critical examination of the observations on which these ideas are based shows, however, that this is not in all these cases the only, or even the most probable, interpretation.

Thus, when it is found, for instance, that the uptake of bromide occurs more readily from a postassium bromide solution than from one of calcium bromide, this does not prove that the permeability of the cell has been increased in the first-named solution or decreased in the latter, for an even more plausible explanation is that when bromide ions are actively taken up, the potassium ions are better suited as accompanying counterions than are calcium ions (see also Chapter 4 of this volume).

Again, when it is found, for example, that the uptake of rubidium ions is specifically depressed by potassium ions, and vice versa, this is no indication of any permeability changes but is more plausibly interpreted as due to mutual competition of the two ions for one and the same carrier (55). Such phenomena will be more fully treated in Chapter 4 of this volume.

Finally, it should not be forgotten that pure solutions of, say, alkali salts tend to injure living protoplasts and thus to decrease their normal diffusion resistance, while calcium ions, for instance, are able to counteract such harmful effects of the alkali ions. In such cases, then, it may be somewhat questionable whether the permeability changes observed should be interpreted as direct effects of the ions on the plasma membrane or perhaps rather as symptoms of a general disturbance of the living system.

It thus seems that there are not very many entirely unequivocal examples left of truly physiological and entirely reversible permeability

changes directly brought about by alkali or alkaline earth ions. But entirely to deny the existence of such influences would, no doubt, be an overstatement.

5. *Oxygen*

In Section III it was stated that a sufficient supply of free oxygen is, in many cases at least, an indispensable condition for effective active transport. Permeability proper, on the other hand, seems scarcely to be affected by anaerobic conditions, provided that they do not last so long a time that the life of the cell is jeopardized. It is often assumed that the existence of osmotic barriers in the protoplast depends on the continued activity of the cell. If this assumption holds true, plasma membranes of anaerobic cells will probably not persist under anaerobic conditions. At the same time, however, the life of the cells will probably be extinguished, and so it may be difficult to decide with certainty whether the abnormal increase in cell permeability is a direct consequence of the anaerobic conditions or whether it is a consequence of the death of the cells. At any rate, oxygen deficiency will tend to increase rather than decrease permeability at the same time as it checks active transport.

6. *Anesthetics*

We have already seen (Section IIIB) that active transport processes may be reversibly checked by anesthesia. However, in the case of true permeation processes the situation is much less clear, for while earlier investigators mostly assumed a similar decrease of permeability in the state of anesthesia, a reversible increase of permeability caused by anesthetics was later convincingly demonstrated in some cases (85b). It would seem then, that, depending perhaps on the special qualities of the anesthetics and on their concentration, both increase and decrease of permeability may occur. There is also the possibility that while the permeability toward some permeators is increased, that toward others is simultaneously decreased. In these respects our knowledge is still very imperfect (cf. 126).

7. *Other Substances*

The literature contains a multitude of statements concerning the alleged influence of different substances on permeability. Thus, to take a single example, the idea that auxins will increase the permeability to water and that they will in this way promote cell enlargement has found some supporters. The experimental evidence in favor of this hypothesis is, however, not very convincing (cf. 117). Other authors

assume that auxins cause a nonosmotic, or active, water uptake, but this assumption, too, rests on a rather weak basis.

8. Nutritional State of the Cells

Hoagland and his associates (70) have shown that the salt-accumulation capacity of root cells is highly dependent on their nutritional state: the less salts and the more carbohydrates the roots contain, the greater is their capacity for salt uptake. Similar observations have since been made on other objects too. But a corresponding influence of the nutritional state on the permeability is not known.

9. Hydration and Dehydration

It is often assumed that a hydration of the plasma membranes will increase their permeability, while a dehydration of them will decrease it. Such an assumption seems in itself plausible, yet it must not be taken for granted that a hydration or dehydration of the bulk protoplasm will always be accompanied by a corresponding change in the osmotically determinative plasma membranes, which differ considerably both in chemical composition and in structure from the bulk protoplasm. Actually we know almost nothing about the hydration or dehydration of the plasma membranes.

10. Stimulation

The ion permeability of nerves and muscles is known to undergo sudden profound changes in connection with the passage of an excitation wave through them. It would therefore not be surprising if some similar change were to occur in plant cells concerned with the conduction of excitations or with the carrying out of sudden contractions. In fact, the sudden drop of turgor occurring, for instance, in the pulvini of *Mimosa pudica* and in the stamens of the *Cynareae* may well be interpreted as due to a sudden increase in permeability causing solutes and water to leak out from the cell sap through the protoplasm. The general occurrence of electrical responses in plants, more or less similar to those in animals, points in the same direction (164). From the standpoint of permeability these phenomena are, however, still rather imperfectly understood.

11. Seasonal Factors

As already mentioned (VIID), a seasonal change from one permeability type to another has in some cases been observed. Perhaps it will be found that the capacity for active transport is even more dependent on seasonal factors.

12. Frost and Drought-Hardening

According to Levitt (110), these cause a considerable increase in the permeability to polar substances, while the permeability to apolar substances remains unchanged.

13. Age

Young cells sometimes differ considerably as to permeability from middle-aged cells, just as these differ from senile ones (26). Probably changes in the capacity for active salt accumulation relative to age and development are even more common. However, as these points will be dealt with in greater detail in Chapter 4 of this volume, they need not concern us here.

14. Injury and Death

It was known even to the earliest students of cell permeability that the approximate impermeability of the protoplasts to anthocyanins, salts, sugars, etc., is characteristic of them only as long as they remain alive, while dead protoplasts are in most cases freely permeable to almost all dissolved substances. Indeed, the striking increase in permeability connected with death has become one of the most commonly used criteria for discriminating between living and dead protoplasts. However, as was pointed out by Overton (125), the increase in permeability does not always occur suddenly: under the influence of dilute formaldehyde solutions, for instance, it occurs gradually in such a way that the protoplasts first become permeable to relatively small molecules and ions and then successively also to larger and larger ones in roughly the following order: (a) alkali chlorides and nitrates, (b) sulfates, phosphates, and tartrates, (c) sucrose, (d) anthocyanins and tannins. Thus the plasma membranes, in such cases, assumed the properties of molecular sieves of gradually increasing pore size.

Osterhout (118) has carried out extensive investigations concerning permeability in relation to injury and death, but unfortunately the method used by him—measurements of the electrical conductivity of disks of the marine alga *Laminaria* sp.—was not very suited to reveal the finer details of the permeability changes in question.

XII. Synopsis of the Permeability Properties of Plant Protoplasts

On the preceding pages, a fairly detailed account of the permeability of plant protoplasts toward different substances has been given. In order to explain as completely as possible the permeability properties of the plasma membranes, we shall try, here, to summarize the prin-

cipal evidence and submit a consistent picture of the problem as a whole.

While the aim is to present the pertinent facts in as unbiased a manner as possible, it is convenient to arrange the data according to two main aspects, namely, (a) correlations between lipid solubility and permeation power, and (b) correlations between molecular size and permeation power.

A. LIPID SOLUBILITY AND PERMEATION POWER

How close is the experimentally found correlation between lipid solubility and permeation power? A great difficulty when we try to answer this question is that we actually know very little about the plasma membrane lipids of plant cells—except what has been inferred from studies on protoplasmic permeability itself. This being so, there is but one way out of this dilemma, namely, to study the solvent properties of some more or less arbitrarily chosen lipoidal solvents, hoping that the solubilities of different substances in some of these solvents will be found to be at least reasonably well correlated with the permeation powers of these same substances.

While it may be argued that this is an empirical method of trial and error, like searching for a needle in a haystack, the situation is not quite as hopeless as it may at first appear. As already pointed out (Section VIIB), all kinds of lipids have certain important solvent properties in common. While we shall often have to speak of "lipid solubility" in a very general sense, we will at least try to be on our guard against drawing unwarranted conclusions from such a flexible concept as this.

Now, how close is the correlation between lipid solubility and permeation power? Or, in view of the fact that a major correlation between these two qualities is already generally recognized, it may be better to formulate the question in a slighly different way: What are the discrepancies found between lipoid solubility and permeation power?

The older literature especially contains numerous statements concerning such alleged discrepancies which are supposed to disprove the validity of the lipoidal theory. It is not possible here to examine each of them separately, but we will try to classify the most important of them into a few categories (a–d) which will be, at least briefly, discussed.

(a) It has been emphasized by numerous writers that living protoplasts must be able to take up several physiologically important, but lipid-insoluble substances, such as sugars, amino acids, and mineral

salts, and it has been suggested that the uptake of these substances is in conflict with the lipoidal theory. In reality, however, this objection is erroneous for two different reasons. First, the often used classification of substances into lipid-soluble and lipid-insoluble ones is, of course, not quite correct, for theoretically all substances are endowed with a certain, although often extremely low, lipid solubility; therefore, no substances are absolutely unable to penetrate lipid membranes by diffusion. Still more important, however, is the fact that the uptake of most "lipid-insoluble" substances is largely due, as we have seen in Section IIIC, to the operation of active transport mechanisms. But such transport processes are superimposed upon permeability per se and thus do not vitiate the correlation between lipid solubility and permeation power proper.

Only in the case of very few, clearly aberrant kinds of cells (*Beggiatoa*, perhaps diatoms also) has a considerable permeability toward practically lipid-insoluble substances been established.

(b) It was claimed, a long time ago, that some basic dyes (methylene green, methyl green, thionine, Methyl Azure) are able to penetrate living cells with considerable rapidity in spite of being insoluble in lipoidal solvents (74, 142). But here we have to note that the alleged "insolubility in lipids" has not been very convincingly established. The method used was to shake an aqueous solution of the dye with a nonaqueous solvent and then simply to see whether the latter had become colored or not. Now there is, first, the possibility that some of the dye bases may be colorless and thus invisible in spite of the dye cations being colored. In such cases one may easily get the false impression that no dye has been taken up by the nonaqueous solvent. Secondly, it must be kept in mind that a distribution ratio of, say, 1:100 or even 1:1000 may be consistent with a fairly rapid penetration into cells. Such a slight lipid solubility is, however, easily overlooked. Finally there is the possibility that the dyes now in question might be almost insoluble in the neutral lipoidal solvents used in the tests but nevertheless distinctly soluble in lipoidal solvents of, for instance, a slightly acidic character (cf. 50, 116). A decision on this question is scarcely possible before the solubility properties of these dyes, and of other dyes too, have been more systematically and more exactly studied than hitherto.

(c) As pointed out in Section VIIC, it has been found that the permeation powers of nonelectrolytes toward *Nitella* cells are not a function of their lipid solubility alone, but also of their molecular size or some similar factor. The same has been found in several other cases, too. These discrepancies between lipid solubility and permeation

power will be discussed below under the heading "Molecular Size and Permeation Power."

(d) Minor discrepancies still exist between the permeation powers of different substances and their solubilities in the lipoidal solvents so far tested, but these can be attributed, at least mainly, to experimental errors and also to the fact that the solvents so far tested (olive oil, etc.) are not identical with the plasma membrane lipids as regards their solvent properties.

B. Molecular Size and Permeation Power

There is not a single nonelectrolyte or weak electrolyte known with a molecular weight below about 45, which does not penetrate cells rapidly. NH_3 (mol. wt., 17), H_2O (mol. wt., 18), HCN (mol. wt., 27), CO (mol. wt., 28), O_2 (mol. wt., 32), hydrazine (mol. wt., 32), methanol (mol. wt., 32), hydroxylamine (mol. wt., 33), H_2O_2 (mol. wt., 34), H_2S (mol. wt., 34), acetonitrile (mol. wt., 41), and CO_2 (mol. wt., 44) may be mentioned as examples of this. Now, most of these substances are fairly, or even strongly, lipid-soluble, too, so their permeation power may, to a considerable extent, be attributed to this fact. A comparison with other substances of similar solubility, however, reveals the fact that the substances mentioned, in all those cases when a quantitative comparison has been possible, penetrate plant protoplasts with a distinctly greater rapidity than would be expected if the permeation constants were proportional to, say $k/M^{1/2}$ or even to k/M, where k denotes the distribution coefficient lipid:water and M the molecular weight.

The influence of the molecular size is most strikingly shown when, within a homologous series, the general rule that the permeation power increases with increasing length of the carbon chain is broken in the case of the first member, or the first two members, of the series. Thus it has been found, in all the cases so far studied, that formamide has a greater permeation power than acetamide, while propionamide, again, permeates more readily than acetamide (31). Similarly ammonia, in at least some cases, seems to permeate more rapidly than methylamine, and this more rapidly than ethylamine, while amylamine, for instance, has a still greater permeation power (6). In the case of urea and the alkylureas, the situation is a little more complicated, for while most plant protoplasts are distinctly more permeable to methylurea than to urea, there exists a minority of cell types which display a considerably greater permeability to urea. On the other hand, in all cases so far studied, except *Beggiatoa*, ethylurea and dimethylurea have been found to have a greater permeation power than methylurea.

The influence of molecular size may also be evident when the permeation power increases throughout a homologous series with increasing length of the carbon chain. For, as pointed out by Wartiovaara (172), the increase in permeation power is not, in such cases, as rapid as the increase in, say, relative oil- or ether-solubility.

So far, we have here been concerned with relatively small molecules. In the case of molecules of medium or larger size, there are few even reasonably exact observations concerning possible correlations between molecular size and permeation power. As already stated, however, it seems that the permeation constants of nonelectrolytes of not too low molecular weight, toward *Nitella* cells, are inversely proportional approximately to the molecular weight raised to the power 1.5, provided, of course, that substances of similar lipid solubility are compared. So far, however, it is impossible to know how general the significance of this observation may be. Besides, even in the case of *Nitella* this rule holds true only as a first approximation. It may therefore well be that the factor actually concerned is neither the molecular weight nor the molecular volume but a hitherto unknown factor which is in some way correlated with them. Moreover, it should not be forgotten that even molecules as large as those of, for instance, neutral red (mol. wt., 252), atropine (mol. wt., 289), cocaine (mol. wt., 303), and thebaine (mol. wt., 313) are, in spite of their molecular size but in conformity with their great lipid solubility, endowed with a very great permeation power toward all the protoplasts yet studied in this respect.

C. Relative Importance of Molecular Size and Lipid Solubility

Although this question cannot be decided unambiguously, the following points suggest that lipid solubility is relatively more important than molecular size. If all we know is the molecular weight, or molecular volume, of a substance, we cannot in most cases predict anything about its permeation power. Thus, substances of the molecular weight of, say, 50, 100, 200, or 400 may equally well belong to the very rapidly as to the very slowly permeating ones. On the other hand, if we know only the distribution coefficient ether:water or, still better, olive oil:water of a noncolloidal substance, we are at once able to anticipate, with reasonable certainty, the order of magnitude of its permeation power. At least from such a practical, or pragmatic, point of view, then, lipid solubility must be regarded as a decidedly more important criterion than molecular size.

This general statement may be supplemented by a more specific example. In Fig. 4 the permeation powers of 70 nonelectrolytes toward *Nitella mucronata* protoplasts are shown [cf. Collander (32), Table 3].

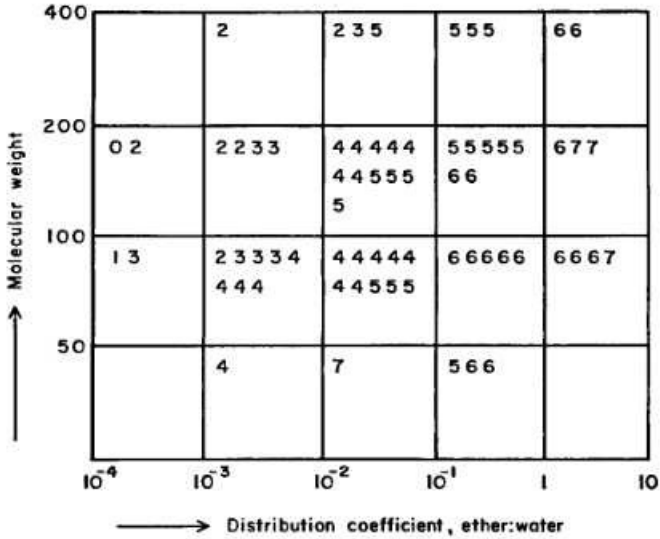

FIG. 4. Permeation powers of 70 nonelectrolytes toward *Nitella mucronata* protoplasts as correlated with their molecular weights and ether : water distribution coefficients. Each figure in the squares represents one permeator. For further details, see the text.

In this graph the relative ether solubilities, used as approximate measures of the lipid solubilities, increase from left to right on the abscissa, while the molecular weight increases upward on the axis of ordinates. In each square the permeation powers of the substances of corresponding molecular weight and ether solubility are indicated in groups or classes according to the scheme shown. (Every substance

Permeation Constants Arranged in Classes									
P (cm/sec)	$<10^{-9}$	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Class no.	0	1	2	3	4	5	6	7	

is represented by one figure in the appropriate square.) On scrutinizing Fig. 4 we find that the permeation powers increase both with increasing ether solubility and with decreasing molecular weight, but at least within the limits of the evidence available the correlation between permeation power and ether solubility is distinctly closer than that between permeation and molecular weight. It is especially evident that when the distribution coefficients lie between, say, 0.1 and 1, or between 1 and 10, even great variations in the molecular weight will not much influence the permeation power. It is also a remarkable fact that

within each square the variability of the permeation power is fairly restricted, i.e., a given combination of solubility properties and molecular size always corresponds to a fairly constant permeation power.

The last-mentioned statement applies not only to *Nitella* cells but to plant protoplasts in general. Thus *if the lipid solubility and molecular size of a substance are known, its permeation power toward most plant protoplasts can be predicted with a high degree of certainty.* It seems appropriate to emphasize this fact, since it is not only theoretically interesting but also, in many cases, of considerable practical importance. There are, it is true, a few cell types (*Beggiatoa, Oscillatoria,* diatoms) whose behavior is somewhat divergent, but these are few indeed as compared with the large number of plant cells endowed with a more or less "normal" premeability.

XIII. Theory of Cell Permeability

A. INTRODUCTION

On the preceding pages an account of the empirically observable behavior of living plant protoplasts toward solutes and water has been given. Theories have so far been avoided in order not to obscure or distort facts. But it is now necessary to explain, as fully as possible, the mechanisms by which certain substances may permeate the plasma membranes, while other substances are, at the same time, more or less prevented from penetrating.

The monographs on permeability by, say, Stiles (157) or Jacobs (93) reveal that, some thirty years ago, a reviewer of the problem of cell permeability had to discuss many different, and often incompatible, permeability theories. No wonder, then, that Jacobs ended his account with the following remarks: "In conclusion, it may be emphasized that what is most needed in the field of cell permeability at the present day is facts. When sufficient accurate quantitative data covering a wide range of material . . . have become available, a satisfactory theory will follow as a matter of course. Until that time, speculations should be reduced to a minimum."

With the growth—both in extent and accuracy—of experimental evidence concerning permeability phenomena, it has now come about that most of the earlier bewildering array of permeability theories have simply died out, so that only two or three theories of cell permeability need now be considered. Even these theories are by no means sharply delimited but merge gradually into one main theory of cell permeability which has several modifications. This main theory embraces several principles referring to different aspects of the permeation process. The

first principle is based on the empirical correlation between permeation power and lipid solubility. Let us call it the lipid-solubility principle. The second, which rests on the correlation between permeation power and molecular size, may be called the molecular-sieve or ultrafiltration principle. Both of these principles are now generally recognized, and the differences between the permeability doctrines of today are thus mainly that some investigators lay more stress on one principle, while others emphasize the other. Moreover, the principles themselves may be formulated and interpreted in somewhat different ways.

What we have to do now, therefore, is not to accept one of these principles and reject the other, but rather to find out how they may be combined so as to interpret actual examples.

B. THE LIPID-SOLUBILITY PRINCIPLE

The correlation between permeation power and lipid solubility, of course, invites the conclusion, already drawn by Overton, that the plasma membranes contain lipids and that substances pass across these membranes by dissolving in lipids. However, against this conclusion several objections (a–c) have been raised.

(a) It has been suggested that it would not be necessary to assume the occurence of lipids in the plasma membranes since, for instance, hydrophobic proteins would also favor the permeation of lipid-soluble, i.e., hydrophobic substances. In itself such an idea may seem plausible, however it should be noted that a differential permeability of protein membranes even remotely similar to that of living protoplasts has never been observed. So far, therefore, it seems most reasonable to ascribe the favored permeation of lipid-soluble substances through the protoplasts to the occurrence of some sort of lipids, or lipoproteins, in the plasma membranes.

(b) Lipid-soluble substances are often also surface-active. Some investigators have therefore assumed that the great permeation power of these substances is not primarily due to their solubility in the plasma membrane lipids, but rather to their adsorption at lipid-water interfaces in the membrane (150). However, the adsorption concept is scarcely applicable to the passage of permeating substances through the plasma membranes unless we assume the existence of water-filled pores in it. The predominance of such pores in the plasma membranes is, however, very questionable, except in a few cases such as *Beggiatoa*. (The pores of the plasma membranes will be more fully discussed below under the heading "The Molecular-Sieve Principle.")

Besides, the parallelism between adsorbability, i.e., surface activity, and lipid solubility is far from complete. The highest degree of surface

activity will be attained if one end of the molecule is pronouncedly hydrophobic while the other end is strongly hydrophilic. The more hydrophobic the molecule as a whole, on the other hand, the greater is the lipid solubility. It should thus be possible to decide between lipid solubility and surface activity as permeation-promoting factors by comparing the permeation power of substances of equal lipid solubility but different surface activity. Such comparisons, however, seem not yet to have been attempted.

(c) Ullrich (163) has stressed that if the plasma membrane is only a few molecules thick and consists of strongly oriented molecules, it would not be correct to speak of the solubility of the permeators in the plasma membrane, since the prerequisite conditions for the validity of Henry's law will not be fulfilled. Bogen (17) has therefore proposed the use of the expression "the effect of intermolecular forces" instead of solubility. Theoretically this seems correct, for, as is well known, it is this strong tendency of hydrophilic substances to form intermolecular hydrogen bonds that tends to keep their molecules in an aqueous phase and prevent them from entering a lipoidal phase. On the other hand, retaining the old "solubility terminology" in this context is not only more convenient but is also factually justified, since the determination of the distribution coefficients is by far the most suitable way of measuring the integrated effect of all the interacting intermolecular forces. Hence we shall continue to speak of solubility in the plasma membrane lipids as a prerequisite for permeation. By this expression we only mean that permeating molecules probably diffuse through the plasma membranes, even if not in true solution in the plasma membrane lipids, nevertheless in a manner which may be most conveniently described as something very similar to a solution process. It may well be that this expression oversimplifies a very complex situation, but, if so, it is at present almost unavoidable, since the physical chemistry of today is unable to provide all of the knowledge necessary to interpret these processes adequately.

Now, what conclusions concerning the chemical nature of the plasma membrane lipids may be inferred from the permeability studies so far carried out?

As mentioned in Section VIIC, the permeation power of nonelectrolytes toward *Chara* cells varies proportionally to the first power of the olive oil:water distribution coefficient, while that toward *Nitella* cells is proportional to a slightly higher power of this coefficient. These observations may seem, at first sight, to indicate that the plasma membrane lipids in *Chara* are about equally as hydrophobic as olive oil, those of *Nitella* being somewhat more strongly hydrophobic. Probably,

however, the situation is a little more complicated. The plasma membrane may consist, at least in part, of more or less rodlike lipid molecules orientated, with their hydrocarbon chains parallel, at right angles to the surface. If so, then there may exist in the plasma membrane a certain zone which behaves almost as a hydrocarbon layer, irrespective of how hydrophilic may be the end portions of these molecules. Probably it is the hydrocarbon-like layer that is the principal barrier slowing down the passage of hydrophilic substances across the plasma membrane. It thus seems possible that it is only this extremely thin layer within the plasma membranes of *Chara* and *Nitella* that is about as hydrophobic as, or somewhat more hydrophobic than, olive oil. The situation in other plant cell plasma membrane lipids is scarcely known even to this meager extent.

Another property of the plasma membrane lipids which may be ascertained by permeability studies is their acidity or basicity. In fact, we have already had cause to assume that the plasma membrane lipids of some plant cells are more acidic, as is seen from their preferential permeability to amides, while those of other cells seem to be more neutral (cf. VIID).

For the arguments favoring the view that phosphatides and sterols occur in the plasma membranes, see Section XIV,A,4.

C. THE MOLECULAR-SIEVE PRINCIPLE

If the permeability of the living protoplasts is compared with that of such artificial membranes as, for instance, the copper ferrocyanide or the collodion membrane, the contrast between them is found to be very striking indeed. By such comparisons we may, then, be tempted to classify the plasma membranes as homogeneous and nonporous. However, closer study of their permeability properties reveals features which render such a classification doubtful.

Danielli (42), applying the theory of activated diffusion to membrane problems, has made an attempt to derive equations which permit a conclusion as to whether a membrane is or is not homogeneous. According to Danielli, if (for slowly penetrating molecules) the quantity $PM^{1/2}e^{2500x/RT}$ when plotted against the oil:water distribution coefficient, or (for rapidly penetrating molecules) $PM^{1/2}$ when plotted against the distribution coefficient, gives a roughly linear relationship, the membrane is probably—as a first approximation—homogeneous. In these expressions P denotes the permeability constant, M the molecular weight, e the base of natural logarithms, x the number of nonpolar groups per molecule, R the gas constant, and T the absolute temperature. Danielli applied these tests to the results of Collander and Bär-

lund (35) on *Chara* and of Marklund (112) on other plant cells and concluded that the membranes of these cells are, as a first approximation, homogeneous lipid layers. It seems, however, that in applying his formulas he had overlooked some rather strongly deviating substances and that, if properly executed, the test would rather indicate some heterogeneity of the plasma membranes. It may be doubted, however, whether the test as applied is inherently reliable (177). Moreover, Danielli himself remarks that the test is difficult to apply in practice. The postulate of the approximately homogeneous character of the plasma membrane was accompanied by the reservation that there are certain small areas on the surface which have special properties and which permit certain types of molecules to enter and leave cells much more rapidly than can be accounted for by diffusion through a homogeneous lipid layer. It is thus unfair to attribute to Danielli such an oversimplification as that implied by the statement that the osmotic barrier of living cells is quite simply a homogeneous lipid layer.

In Section VIIC it was shown that the permeation power of nonelectrolytes toward *Nitella* protoplasts is inversely proportional approximately to the molecular weight raised to the power 1.5 (granted equality of lipid solubility). In itself this implies no more than a fairly high viscosity of the plasma membrane. What is more important, however, is that the very smallest molecules display a distinctly greater permeation power than would be expected on such an assumption. In other words, while the coefficients of free diffusion increase steadily with decreasing molecular size, the permeation power increases rather abruptly when the molecular weight decreases below about 50. This has been observed not only in *Nitella*, but for several other cells, too.

The simplest explanation for these observations is that the plasma membranes contain some sort of pores which will allow the smallest molecules to pass but are impervious to larger molecules. The plasma membranes thus seem to act simultaneously as selective solvents and as molecular sieves. This, admittedly rather general, view has been called the lipid-sieve hypothesis (134). It has been defended by the present author and his co-workers, among others (8, 29, 31, 32, 35, 52, 112).

However, if this view is accepted, great care should be taken in speaking about the assumed "pores" of the plasma membranes. Our familiar, macrophysical concepts are too crude to be applied here. In fact, the widespread opinion that the pores are permanent, water-filled channels traversing the membrane can scarcely be correct. For, as pointed out by Wartiovaara (172), if the permeating molecules could actually take either of two alternative routes, namely, solution in the lipids and movement through the pores, then the total permeation would equal

the sum of the "lipid permeation" and the "pore permeation." If so, the smaller the permeation through the lipids, the more the pore permeation would predominate. In reality, however, this is not so: the pore permeation seems actually to be about as distinct in, say, the series of the monohydric alcohols as in that of the fatty acid amides, although the members of the first-named series have a much greater lipid solubility than those of the latter. Moreover, the great temperature coefficient values of the permeation processes also indicate that these processes do not consist of diffusion through permanent water filled pores.[5]

We are thus led to assume that in the great majority of plant plasma membranes there are no persistent, or mechanically fixed, water-filled pores. But, on the other hand, it really seems that there exist some kinds of plant protoplasts which must be assumed to be provided with such pores. This applies especially to *Beggiatoa*, whose permeability properties are difficult to explain without such an assumption. The pores of *Beggiatoa* must even be of considerable width, many of them letting through sucrose molecules, for instance. Danielli (42) has presented some calculations which indicate that either *Beggiatoa* cells possess an extremely small number of wide water-filled pores or else the diffusion-resisting layer is much thicker than are ordinary plasma membranes. The latter alternative seems decidedly more probable for, after all, the whole visible plasma layer and the whole cell wall should in this case also be understood as belonging to the "diffusion-resisting" layer. Moreover, Wartiovaara (172) has pointed out that the peculiar permeability properties mostly ascribed to the diatoms can be understood if it is assumed that about 3% of the area of their plasma membranes is of the *Beggiatoa* type, while the rest is of the usual type.

But how is the apparent sieve effect of the plasma membrane, i.e., its preferential permeability to the smallest molecules, to be explained in the case of membranes devoid of permanent, water-filled pores?

One possibility is that the osmotically determinative zone of the membrane is built up of regularly orientated, long lipid molecules, say, in a bimolecular layer (cf. Section XIV). Of course the plasma membrane may also contain layers of protein molecules etc., but these may perhaps be neglected here if we attempt only to obtain a simplified picture of what is responsible for the sieve effect. In a structure of this

[5] Ussing (166) has recently proposed a criterion by which he believes it possible to decide whether or not plasma membranes contain water-filled pores. If the water permeability of a cell membrane as measured by the rate of osmosis proves higher than its water permeability as measured by the rate of diffusion of isotopic water, then it seems to him appropriate to speak of a bulk flow of water through pores in the membrane. However, whether plant cell membranes are equipped with pores in Ussing's sense seems not yet to have been investigated.

kind, and owing to their incessant thermal vibration, the lipid molecules of the membrane will from time to time leave small gaps between each other. Each such gap will, of course, exist only for a very short time and then close again. These gaps will permit the passage of solute molecules more or less easily, depending principally on two factors, (a) their affinity for water, on the one hand, and to the plasma membrane substance on the other, and (b) the dimensions of the gaps and of the permeating molecules.

Factor a: It is clear that the more hydrophilic the permeating molecules, the more difficult will it be for them to become detached from the aqueous phase outside the plasma membrane and the greater the kinetic energy necessary to cause entry to the lipid phase. More precisely stated: the minimum energy required to enter the lipid phase is inversely proportional to the lipid:water distribution coefficient of the permeator. (Concerning the membrane as a "potential energy barrier" cf. Section IVB.)

Factor b: Even if the kinetic energy of the permeating molecule, at the very instant of contact with the membrane surface, equals or surpasses the minimum energy required, it is not certain that it will penetrate into the membrane. This will happen only if there is a hole of suitable dimensions precisely at the point of impact. Now, the occurrence of small holes is, of course, more likely than that of larger ones. This may perhaps suffice to explain the preferential permeability to small molecules. It is, however, conceivable that, strictly speaking, it is not the molecular size, or even the molecular volume, as such, that is decisive, because the steric properties of the permeating molecules may also be of importance.

Wartiovaara (172) has discussed in somewhat greater detail one such comparatively simple case—that of rod-shaped permeator molecules. He supposes that these molecules are able to penetrate the lipoidal membrane only if, on striking the surface, they are orientated approximately perpendicularly to its surface or if, before the hole has shut, they have time to achieve this orientation (cf. Fig. 5 which gives a schematic picture of what may happen). Starting from these premises, Wartiovaara reaches the conclusion that with increasing length of the permeating molecules, and thus also with increasing slowness of their reorientation, their orientation at the moment of striking the surface will concurrently become a more and more important factor reducing the probability that a molecule will actually penetrate. In other words: the greater the length of the permeator molecule the more exactly perpendicularly to the membrane surface must it be orientated in order to be able to penetrate. Wartiovaara found this theoretical postulate

corroborated by experiments concerning the permeability of *Nitella* cells toward water and primary alcohols. In these experiments it was observed that, other things being equal, the chance for penetrating the plasma membranes is inversely proportional to the transverse moment of the inertia of the permeator molecule. (The magnitude of the transverse moment of the molecular inertia was calculated from the atomic weights and the mutual distances between the atoms in the molecule.) Quite similar results were obtained by Äyräpää (5) in his study of the permeability of yeast cells to ammonia and amines (cf. Section IXC).

In the case of molecules of a more complicated structure, no such quantitative calculations as those carried out by Wartiovaara and

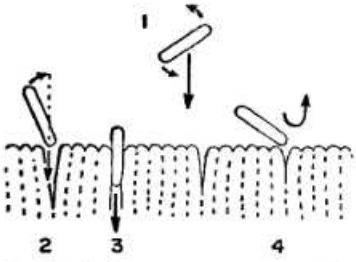

FIG. 5. Diagrammatic sketch of the penetration of rodlike solute molecules into a plasma membrane consisting of parallelwise orientated lipoid molecules. Molecule *1* is in free whirling motion in the aqueous medium surrounding the cell. Molecule *2* is just striking the plasma membrane and will, owing to its fairly favorable orientation and also to the existence of a hole in the membrane in the right place, probably be able to penetrate the membrane as molecule *3* is just doing. Molecule *4*, on the other hand, strikes the membrane in such an unfavorable orientation that it will be jostled back into the medium. From Wartiovaara (172).

Äyräpää have so far been possible. However, in experiments with *Nitella* (32) it has been found that richly branched, and thus bulkier, molecules, such as those of *tert*-butyl alcohol, triacetin, and trimethyl and triethyl citrate, all seem to permeate somewhat more slowly than their lipid solubility and molecular weight alone would lead one to expect.

To sum up, the old question of whether or not the plasma membranes are homogeneous now seems to be largely a question of definition, for the plasma membranes are intermediate between distinctly porous membranes, on the one hand and truly homogeneous ones, on the other. Their pronouncedly preferential permeability to the smallest molecules is suggestive of the molecular-sieve concept here. But, on the other hand, most plasma membranes are probably devoid of fixed and permanent water-filled pores and hence, from this point of view, they

can behave as though homogeneous. This statement does not imply, however, that they are devoid of internal structure. On the contrary, some sort of anisotropic structure is almost certainly characteristic of these membranes and will, no doubt, influence their permeability properties. The details of this internal structure are, however, still obscure. Before they can be worked out, it will be necessary not only to submit the plasma membranes themselves and their permeability properties to a more thorough study, but also to investigate more closely the diffusion phenomena occurring in artificial membranes of molecular dimensions consisting of anisotropically orientated lipid molecules, so as to give a sound physicochemical basis for the understanding of the peculiar permeability properties of such systems.

D. Successive Steps in the Permeation Process

The permeation of a substance across a more or less homogeneous plasma membrane consists of three successive steps: the penetration through the water-plasma membrane interface, the diffusion through the interior of the plasma membrane, and the movement from the membrane into water again. Now, which of these steps will determine the rate of the over-all permeation process? This problem was first taken up by Danielli (42) but was later more thoroughly treated by Zwolinski *et al.* (177). According to them the rate-determining step is in some cases the diffusion within the membrane, in other cases the diffusion through the solution-membrane interface. The authors stress, however, that the data so far available are too inadequate to give a conclusive answer to this question.

These workers also calculated that the values of the diffusion coefficients for nonelectrolytes in the plasma membranes are of the order of 10,000 to 100,000 times as small as their values for diffusion in aqueous solutions. This is considered to be primarily due to the higher energies of activation for diffusion in the membranes. The diffusion coefficients as thus calculated "occupy an intermediate position in the spectrum of diffusion constants in solids and liquids which bespeak a semisolid structure for natural membranes." It seems, however, that these calculations, also, are all of a rather preliminary nature.

XIV. Other Evidence on the Composition and Structure of the Plasma Membranes

A. Different Lines of Approach

The concept of the plasma membranes which we have arrived at on the basis of our analysis of their permeability properties consists prin-

cipally of the following points: (a) These membranes contain, as essential constituents, various lipids more or less resembling olive oil as regards their solvent properties. (b) The plasma membrane lipids are more acid in some cells, more neutral in others. (c) The structure of the plasma membranes is such that it enables them to behave to a slight extent as a kind of molecular sieve. (d) Permanent, water-filled pores can hardly be thought, however, to occur in the plasma membranes, except in a few exceptional kinds of cell. Finally, several active transport processes seem to imply the occurrence of certain enzymes in the plasma membranes.

Now this, of course, is only a very crude and a very imperfect picture, and it therefore seems necessary to examine whether there are any other lines of approach by which our concepts of the qualities of the plasma membranes could be supplemented and made more precise. One considerable difficulty in this connection is that these membranes are so extremely thin that they cannot be differentiated from the bulk protoplasm by microscopic means. Even the electron microscope has not so far revealed much of their structure, although some recent electron microscope measurements indicate that the tonoplasts of *Nitella* and *Vaucheria* have a thickness of about 70–100 A, while the plasmalemma is believed to be slightly thinner (64b). There are, however, some other modes of attack which have already contributed considerably to our knowledge in this field.

1. Micrurgical Evidence

Among the micrurgical experiments which have aided in elucidating the properties of the plasma membranes, those of Plowe (133) and of Chambers and Höfler (27) are especially noteworthy. In both cases the epidermal cells of *Allium cepa* were principally studied. Plowe found that when a strand is pulled out from the surface of the plasmolyzed protoplast by the microneedle, both mesoplasm and plasmalemma will at first follow the needle and cannot be distinguished from one another. Gradually, however, the mesoplasm rounds into droplets, while the plasmalemma persists as a slender thread connecting the droplets and forming a layer over each of them (Fig. 6). The conclusion that the outer layer and the inner cytoplasm are distinct seems inescapable, for although viscous fluids such as tar could no doubt also be pulled out into long, slender threads, there would in this case be no droplets on the thread. Tearing experiments with the microneedle also showed that both plasmalemma and tonoplast are elastic fluids, thus indicating that they do not consist solely of lipids but must also contain some fibrillar elements, probably proteins.

Chambers and Höfler (27) stress that the tonoplast membrane is a highly cohesive and extensible fluid film. Its cohesiveness is such that a glass microneedle can be passed readily through it without causing rupture. The membrane simply closes over the moving needle and remains intact. The highly fluid nature of the membrane is strikingly shown by the fact that a strand, extending to the tip of the needle, can be made, at its base, to slip along the surface of the tonoplast at right angles to the long axis of the strand. On coming into contact with the water-air interface, the tonoplast spreads out and disappears, leaving behind no appreciable trace of material. The lipoidal nature of the tonoplast is indicated *inter alia* by the observation that a droplet of liquid

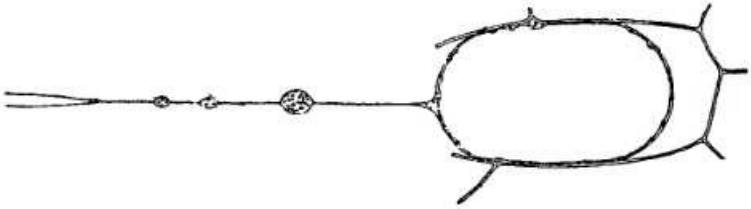

FIG. 6. Strand of protoplasm pulled out from the surface of a plasmolyzed protoplast by the microneedle. From Plowe (133).

paraffin or olive oil snaps on and adheres so strongly to the tonoplast that it cannot be removed without rupturing the membrane or carrying a portion of the tonoplast away with it.

2. *Surface-Tension Measurements*

Some important hints as to the composition and structure of the plasmalemma have also been gained from studies of its surface tension (66). The most remarkable finding is the smallness of this tension (about 0.1–2 dynes per centimeter) as compared with that of oil drops in water (about 8 dynes per centimeter). This shows at once that the cell surface cannot consist purely of lipids. On the other hand, Danielli and Harvey (45) have pointed out that the smallness of the tension at the cell surface is understandable if we assume that there is a layer of protein adsorbed on the lipid film. In fact such a layer would at the same time account both for the smallness of the surface tension and for the striking elasticity of the plasma membrane.

3. *Chemical-Resistance Studies*

Another method by which the outer plasma membrane may be studied is to watch its destruction by substances known not to penetrate

into the mesoplasm, and which therefore act solely on the plasmalemma. Except in the case of the red blood cells (cf. 46, 135) this approach has not so far been much used, but it is to be expected that closer analysis of the ways in which the plasmalemma may be destroyed will add considerably to our knowledge of its original structure and composition. Moreover, this is a method of long standing, for in his classical paper of 1899 in which the lipoidal theory of permeability was first enunciated, Overton already pointed out that the lipid impregnating the plasma membranes can scarcely be a fat in the strict sense of the word, since algal filaments can be kept immersed for several days in dilute sodium carbonate solutions without showing any sign of injury; by such treatment fats, if present in the plasmalemma, would probably be saponified. The fact that numerous algal cells are very resistent even to fairly concentrated sodium carbonate solutions (pH about 11.8) has since been amply verified by Höfler (83), but the cytological consequences of this fact are so far not quite clear since there seems to be no comparable investigation revealing the saponification rate of different fats under the influence of such solutions.

According to Ballentine and Parpart (7), neither tryptic nor peptic enzymes affect the permeability of red blood cells, while lipolytic enzymes cause an increase of their permeability, presumably by splitting off one of the long-chain fatty acids of the phospholipoids which are present in their plasma membranes. Comprehensive experiments of this kind with plant cells are still lacking. Mothes (113) incidentally observed, however, that the tonoplasts of the alga *Sphaeroplea annulina* are destroyed by papain. He therefore concluded that proteins constitute an essential part of them.

4. Comparisons with Blood-Cell Ghosts

When red blood cells are hemolyzed, i.e., caused to give off their hemoglobin, very thin membranes are left which are supposed to be plasma membranes, free of intracellular material. These so-called "ghosts" constitute an easily obtainable and thus very attractive material for studies on the properties of plasma membranes. For instance, the ghosts have been subjected to careful chemical analyses (cf. 129). The results, show that they consist of relatively few substances—besides an unknown amount of water—namely, of lipids (both cholesterol and different phosphatides) and proteins. The lipid:protein ratio shows a remarkable constancy, varying only, in the case of the 17 different mammals studied, between 1:1.4 and 1:1.8 by weight. This would mean that there are some 70 lipid molecules for every protein molecule present in the membrane. The bulk of the lipids is, however, not free

but occurs as lipoprotein complexes in which some of the lipid component (cholesterol) is more loosely, and some (phosphatides) more strongly, bound to the protein. Lipo-carbohydrate-protein complexes have also been observed in the ghosts.

The thickness of the ghost membranes has been evaluated in several different ways. The results are somewhat variable, but the most reliable values attained seem to be of the order of 50–100 A, corresponding to about 2–4 lipid molecules. Besides, the variation encountered may be due in part to the fact that some of the methods used will reveal the thickness of the lipid layer only, while others probably give the thickness of the total ghost membrane.

All these observations made on blood-cell ghosts are of considerable interest to the student of plant cell membranes, too. But, on the other hand, it should not be forgotten that the red blood cells are highly specialized cells, and thus what is true of their membranes may not necessarily be true of, for instance, plant cell membranes.

B. CONCLUSIONS

There exist, as we have seen, numerous observations which suggest that the plasma membranes of plant cells consist of lipids and proteins and/or lipoprotein complexes. But how are these components arranged in relation to each other?

Frey-Wyssling (60) assumes that the plasma membranes consist of globular protein molecules and that the interspaces between them are more or less filled with lipid molecules. The lipid filling is thought not only to explain the impermeability of the membrane toward lipid-insoluble substances but also to act as a stabilizer preventing the denaturation of the protein component. The question of the extent to which the lipid molecules are free or only loosely attached to the protein molecules and to what extent more or less stable lipoprotein complexes exist has not been definitely decided by him.

A more elaborate concept of plasma membrane structure has been presented by Danielli (42–44). According to him, the greater part of the plasma membrane consists of a roughly bimolecular lipid layer covered on both sides by adsorbed protein molecules, as shown in Fig. 7. The lipid molecules (at least the outermost of them, if there are more than two layers) are orientated perpendicularly to the membrane surface so that the hydrated polar groups are in the oil-water interfaces, while the hydrophobic tails are directed inward. The lipid layer has a fluid character, and thermal agitation will continually give rise to openings which are then shut again. How far the protein is mechanically superimposed upon the liquid, and how far the surface must be

regarded as a specific protein-lipid complex, is undecided. The adsorbed protein molecules are regarded as denatured (possibly reversibly denatured). They "consist of polypeptide chains, or meshworks of such chains, lying in the plane of the interface [Fig. 8], with the hydrocarbon portions of the amino-acid residues dissolved in the lipid layer and the polar groups in the aqueous phase. There may be a further layer of globular protein adsorbed on to this primary layer. . . . The mechanical properties of the adsorbed polypeptide chains are probably largely responsible for the elasticity and relatively great mechanical strength of the plasma membrane. A single polypeptide chain may be 50 mμ or more in length and will be attached to similar chains by

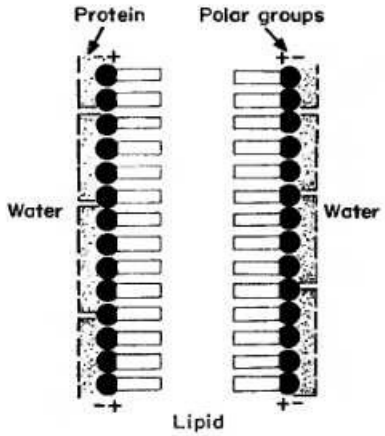

FIG. 7. Diagram of plasma membrane consisting of a lipid layer on each side covered with a layer of adsorbed protein molecules. From Harvey and Danielli (67).

hydrogen bonds and other linkages and will thus 'tie' the various parts of the membrane together, but, owing to the elastic properties inherent in such interlinked chains, without conferring upon the membrane an undesired brittleness." [Danielli (43), pp. 152–153.] Moreover, while the continuous lipid layer described above is supposed by Danielli to be the basic pattern of the plasma membrane, there may be relatively small areas, or patches, which permit abnormal permeation of special substances (e.g., glycerol in the case of certain erythrocytes).

In a long series of papers Bungenberg de Jong and his school [see Booij and Bungenberg de Jong (20)] have developed the hypothesis that the plasma membranes consist essentially of phosphatide double films which contain certain amounts of such substances as cholesterol and phosphatidic acid. Such a film will form a complex with protein

and inorganic cations (Fig. 9). It will be noted that the coherence of such a film is secured not only by London-van der Waals' forces acting between the hydrocarbon chains but also by Coulomb forces between the ionized groups. Such a film will therefore have a considerable

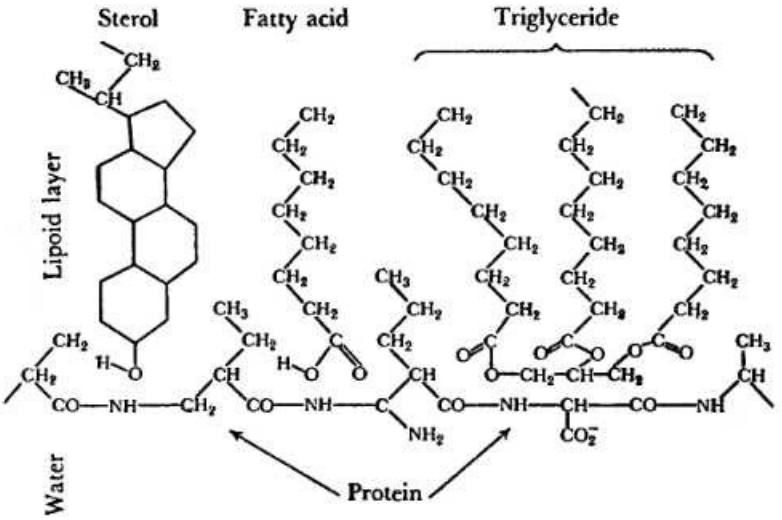

FIG. 8. Details of chemical structure of plasma membrane consisting of regularly orientated lipid molecules and covered with a layer of adsorbed protein molecules. From Danielli (42).

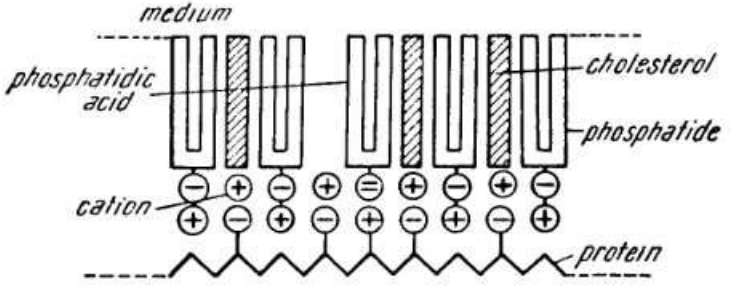

FIG. 9. Diagrammatic representation of plasma membrane consisting of phosphatides, phosphatidic acid, cholesterol, protein, and cations. From Booij and Bungenberg de Jong (20).

stability and will readily be formed spontaneously from constituents present in the cytoplasm. It is stressed by Booij and Bungenberg de Jong that although their models, when drawn on paper, may look very static, the molecules of the membrane will actually be in incessant

thermic agitation. This will give rise to the appearance of smaller and larger holes between the carbon chains of the lipids. So it will be understood that such a membrane will act as a filter and as a selective solvent at the same time. The effect of temperature on the agitation of the membrane molecules also accounts for the high temperature coefficients of the permeation of large hydrophilic molecules. Moreover, variations in the amounts and properties of the membrane components will readily account for the variations found in the permeability of different cell types and there will be no difficulty in explaining permeability changes brought about by various substances. Booij and Bungenberg de Jong even suggest mechanisms enabling the active transfer of substances across such membranes.

In conclusion, it may be stated that while the details of the plasma membrane structure are still more or less hypothetical, the more gross features of their structure are now fairly well agreed upon. At least there exists a basis for continued research.

XV. Role of the Plasma Membranes in the Life of the Plant

During the two or three first decades of the present century there was a widespread tendency to think of living cells as if they were simply aqueous spaces isolated from their environment by selectively permeable, but inert, membranes. Today we realize that this "collodion bag concept" was a flagrant oversimplification. The principal defect of this mode of thought was, of course, that it totally neglected the active transport processes. That this really was a serious deficiency has become increasingly evident as the role of active transport has been appreciated.

It is also regrettable that there has been so much reference to the permeability of the plasma membranes, while their impermeability or, more properly speaking, the very great resistance they offer to the diffusion of many substances has been relatively neglected. And yet it is obvious that one of the most important functions of these membranes is to act as barriers which permit the undisturbed functioning of the highly susceptible living machinery by isolating it, on the one hand, from the cell sap and, on the other, from the fluctuating environment of the cell. It seems, in fact, plausible to assume that it is largely due to their effective isolation from the surrounding medium that living cells are able to inhabit such a variety of milieux displaying a very great variability as to pH, salt concentration, etc. The development of molds even in concentrated solutions of cupric sulfate may be cited as an impressive example of the remarkable independence of living cells of the chemical composition of their surroundings. Also the turgor

of plant cells, so important for the normal elasticity of the plant body, would not be possible if the protoplasts were not almost impermeable to the salts, sugars, and other normal constituents of the cell sap and yet at the same time permeable to water. As was pointed out by Danielli, the effective isolation of the protoplasts also makes it conceivable that viruses are probably able to infect a plant only when they can reach the protoplasm through the surface of a damaged cell and that infection of healthy cells probably occurs via the protoplasmic connections between adjacent cells.

On the other hand, the isolation of the protoplasts is far from perfect. Thus there are numerous poisons which, either owing to their lipid solubility or to the smallness of their molecules, are able to penetrate all protoplasmic membranes. Ethyl alcohol, acetic acid, and also lactic acid may be cited as examples of toxic substances produced by living cells and endowed with such a great penetration power that no protoplast is able to prevent their entry. The same holds true of numerous poisons, less frequently met with under natural conditions, such as ether, chloroform, and other anesthetics, and also phenol, hydrocyanic acid, carbon monoxide, sulfur dioxide, hydrogen peroxide, formaldehyde, chlorine, bromine, iodine, and bichloride of mercury. (The last-mentioned substance is not a very strong electrolyte; its lipid solubility is considerable.) Also the highly lipid-soluble phenoxyacetic acids, etc., used for weed control, may be mentioned in this context. On the other hand, there are several poisons which penetrate protoplasts to a considerable extent only after destruction of the plasmalemma. Sodium and potassium hydroxide, hydrochloric, nitric, and sulfuric acids, potassium permanganate, and potassium chromate are examples of such substances.

If we then proceed to consider the uptake of nutrients by the cells, we observe that water, carbon dioxide, oxygen, and ammonia are examples of nutrients having such a great penetration power that their active uptake would seem superfluous. On the other hand, there is, as we have already seen, an ever-increasing body of evidence in favor of the view that salts especially, but probably also sugars and amino acids, are, preeminently, actively absorbed.

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