

A. S. TROSHIN

PROBLEMS OF  
Cell Permeability

REVISED AND SUPPLEMENTED EDITION

TRANSLATED BY

M. G. HELL

TRANSLATION EDITED BY

W. F. WIDDAS

*Professor of Physiology in the University  
of London, Bedford College*

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## Foreword by the Scientific Editor

IN EDITING the English translation of Professor Troshin's book I have tried to maintain the Author's own style of writing, his marshalling of the experimental material and his interpretations of it, so that this book should represent in scientific English a faithful translation of the Russian text.

Workers in the permeability field cannot fail to be impressed by the breadth of Professor Troshin's interests and scholarship. His citations from the literature, covering, amongst others, publications in Russian, German, French, English and American journals, are more comprehensive than in any similar text.

Although some of his views and interpretations are "controversial" and do not coincide with those currently held by other authors, his work gives a timely reminder of the complex issues involved and serves as a caution against an oversimplification of the permeability problems which are still to be solved.

W. E. WIDDAS

## Author's Preface to the English Edition

THE problem of cell permeability is one of the most important and urgent problems in the physiology of the cell. It has been the subject of intensive study in many countries. In the U.S.S.R. much detailed and original work has been done on the important questions raised. However, it must be stated with regret that this work is, as a rule, unfamiliar to scientists in other countries and is not quoted in their publications. This is, evidently, to be explained by the relative unavailability for foreign scientists of the scientific literature published in our country. It is my burning hope that the publication of this book in English will enable foreign scientists to become more closely acquainted with the work on the permeability of cells that has been published in the U.S.S.R.

Summarising the researches made in the sphere of cell permeability is a very difficult task because of the enormous amount published on the question and the abundance of hypotheses made to explain the experimental results obtained. In this respect the task is made a little easier by the books that have been published to date and the comprehensive reviews of the topic (Gellhorn, 1929; Brooks and Brooks, 1941; Davson and Danielli, 1943; Höber, 1945; Harris, 1960). Furthermore, the writers mentioned completely fail to give any exposition of the work done in our country.

In the monograph *Problems of Cell Permeability* now offered to the reader both old and new theories of cell permeability are expounded and the sorptional theory of the permeability of cells is argued in detail. The book was published in Russian in early 1956, being written in the course of 1953–4. It was published without substantial change or supplementation in German in 1958 and without change in Peking in 1961 in Chinese. For the English edition the book has been completely revised. Almost two-thirds of the text has been rewritten afresh, with the addition of a substantial amount of new information. The review of the basic literature on the problem has been brought up to the end of 1961.

An obvious deficiency of the present book is the inadequate treatment of the material on the permeability of plant cells and the absence of chapters devoted to the permeability of cell organoids and pinocytosis. The author was unable to realise his intention of making good these gaps because the revision of the book has in any case extended to almost two years because of his preoccupations with other matters.

The initiative for the publication of my book in English comes from Professor Z. M. Bacq (of Belgium), for which I should like to express my deep

gratitude to him. I should also like to thank the management of the Pergamon Institute and the Pergamon Press for undertaking the publication. Nina A. Vinogradova, a colleague in my laboratory, has given invaluable help in the preparation of the manuscript of the book for the English edition for which she has my heartfelt gratitude.

A. S. TROSHIN

Leningrad

Institute of Cytology of the Academy of Sciences  
of the U.S.S.R.

## Preface to the Russian Edition

THIS book by A.S. Troshin is devoted to a problem of the greatest importance both for the theory of physiology and for practical medicine and agriculture, the problem of cell permeability. This is the first full-scale work written by a Russian author on the subject. Its value lies not only in that the reader will find in it an exhaustive review of the latest work on the problem, but also in that the author here lays the foundation of a new conception of cell permeability.

For the last 70 years, as is well known, the so-called "membrane theory" has reigned in the study of cell permeability. This theory lies at the foundations of contemporary ideas about the penetration of substances into the cell, the nature of bioelectric potentials and of local and widespread excitation, and so on.

Some workers, for example, M. Fischer and V. Lepeschkin, have tried to criticise the membrane theory, but without success: the theory has remained, until recently, generally accepted.

For many years my colleagues and I have been doing research in the sphere of cell permeability. As a result of the data we have ourselves obtained and also from our analysis of the work of others, we have come to be very sceptical about the membrane theory. We have come to the conclusion that this theory gives a completely false idea of the structure of the cell and the nature of the substances contained in the protoplasm. Furthermore, as a result of the apparent simplicity of the scheme it offers in explanation of many problematic phenomena, the membrane theory has acquired great popularity among physiologists and has, in our opinion, induced them to follow a false line in their theoretical researches. The publication of our studies on permeability aroused a controversy, in which A.S. Troshin's present book is the last word.

It should be remarked that the critical side has been predominant in our work on permeability. We have been collecting references and analysing the literature which demonstrates the incompetence of the membrane theory. We have ourselves been testing many of its propositions and discovering their inaccuracy in a series of fundamental questions. As regards the positive, constructive side, we have, till lately, been compelled to confine ourselves to guesses and hypotheses.

We have said that in explaining the various phenomena of penetrability, the principle of selective diffusion through hypothetical membranes must be

replaced by the principle of the distribution of substances between the protoplasm and the surrounding medium. We have supposed that such a distribution is achieved thanks to the fact that the protoplasm water has the properties of a phase in relation to the water surrounding it. We have not, however, been able to say anything definite about the nature of this phase, and this has been a limitation of our case, to which our opponents have rightly pointed.

A. S. Troshin's great service is that in his book he gives a completely concrete answer to the question of the phase nature of the live protoplasm. On the basis of a series of very convincing experiments he comes to the conclusion that those features of the distribution of substances between cells and the medium, which earlier workers attempted to explain by means of the membrane theory, could be understood on the basis of the fact that the protoplasm is a coacervate system in which all the water is connected with the protein component of the protoplasm and, therefore, has different solvent properties in comparison with the free water of the medium.

A. S. Troshin also showed that the ability of the protein component to adsorb and chemically bind dissolved substances allows the live protoplasm to concentrate them within the cell as much as it needs, while the sorption level of the proteins depends on the physiological state of the protoplasm and can vary owing to the active participation of metabolism, which thus regulates the accumulation of substances in a live cell. Finally, A. S. Troshin sets out the method he has developed for determining that part of a substance which penetrates within the protoplasm and is freely dissolved in its water and that part which is in the bound state.

As may be seen from what has been said above, A. S. Troshin's book is a major original work; in addition, the full review of the literature on the problem of permeability to be found here makes it a valuable reference book.

We have no doubt that this book will be of interest to a wide circle of biologists.

D. NASONOV

## Introduction

THE problem of cell permeability deals with the questions connected with the laws of the entrance of substances from the surrounding medium into cells and the excretion from the latter of the products of intracellular metabolism. Consequently, it is directly related to metabolism and forms part of the very important general biological problem of the interrelation of an organism with its medium.

This problem has been studied for more than seventy years, and at present scientific interest in it, so far from decreasing, is ever on the increase. This is readily intelligible if it is remembered that the data characterising the permeability of cells are widely used in various branches of biology and medicine. To date, however, research workers have sharply divergent opinions about the fundamental questions of cell permeability.

The conception ruling at the present time is the membrane theory; as is well known, the basis of this is the idea that any animal and plant cell behaves like an osmometer. Advocates of the old (classical) membrane theory assert that almost all the water in the protoplasm does not differ in its physico-chemical characteristics from the water of the surrounding medium and is an ordinary solvent and that all the fundamental mineral substances forming part of the composition of the protoplasm in it are in the dissolved state and are completely ionised. It is supposed that all live cells have on their surface a special layer (plasmolemma, protoplasmic or cell membrane), a barrier separating the contents of the cell from the surrounding medium. This barrier is semipermeable in character: like an osmometer membrane, it is freely permeable to water molecules, but water-soluble substances pass through it only with difficulty or not at all.

After the formulation of the fundamental propositions of the membrane theory, it quickly grew into a conception of wide application in general biology. Not only were the phenomena of cell permeability and its functional changes connected with the degree to which substances passed through the semipermeable membrane: a number of other properties of live systems were connected with this too, for example, the regulation of metabolism, the irritation of cells, excitation and its conduction, the origin of bioelectric potentials, narcosis, the difference in the concentrations of individual substances in the cell and in the medium, the immiscibility of the protoplasm with the water of the surrounding medium, and so forth.

Soon, however, the membrane theory encountered difficulties which it was unable to overcome. Already at the beginning of this century several distinguished physiologists came out against this theory, pointing out that its fundamental propositions were erroneous. It was established by many investigators that all those substances which, according to the membrane theory, should not penetrate the resting cell (carbohydrates, amino acids, mineral ions of strong acids and alkalis, etc.) in fact penetrate various animal and plant cells. It was also found that animal and non-vacuolated cells do not behave like osmometers. In this connection there has recently appeared a series of new theories, whose authors strive by modernising the old ideas about the structure and properties of the protoplasmic membrane to explain this wealth of experimental material that cannot be fitted into the narrow frame of the classical membrane theory. But in most cases these new theories are speculative constructions and do not rescue the membrane theory from the impasse in which it remains at the present time.

Comparison of the facts relating to cell permeability with the material characterising other facets of the activity of cells demonstrates convincingly that the present need is not for the improvement of some or other link in the membrane theory, but for its complete revision.

The membrane theory of cell permeability must be replaced by another, more refined theory based on different principles. The foundations of such a theory were laid already many years ago by the work of many scientists.

The first to subject the membrane theory to searching criticism were Fischer and Moore (1907), Roaf and Alderson (1907), Moore and Roaf (1908, 1913), Moore, Roaf and Webster (1912), etc. These authors considered the water exchange of cells, and also the absorption by cells of mineral and organic substances out of the medium, not from the point of view of the laws of osmosis and diffusion, but from the point of view of colloido-chemical processes taking place in the protoplasm itself.

The "no-membrane" theory of cell permeability was further developed by Lepeschkin (1924, 1928, 1930, 1936), Fischer and Suer (1935, 1938, 1939), Nasonov and his colleagues (Nasonov, 1939, 1949a; Aleksandrov, 1939a; Nasonov and Aleksandrov, 1937, 1940, 1943; Kamnev, 1938; Aizenberg, 1939), Ling (1952, 1955, 1960), Ungar (1957, 1959) and other workers.

These authors attribute the fundamental role in the phenomena of cell permeability to the whole mass of the live substance. On the basis of a vast quantity of data, they assert that the protoplasm of live cells is a fluid which differs fundamentally in its physico-chemical characteristics from the surrounding aqueous medium.

According to Fischer, there is absolutely no free water in the protoplasm—all the water molecules form part of special complex organic compounds of which the live substance is composed. According to Lepeschkin, the proto-

plasm is a "fluid"—something like a fluid consisting of loose chemical compounds of proteins with lipoids (lipo-proteids or "vitaids"). And it is in these "vitaids" that the protoplasm water is dissolved. Nasonov and Aleksandrov (1943) also came to the conclusion that the live substance is not a solution of organic and mineral substances in water, but a fluid of a special kind, whose dispersion medium behaves like a phase in relation to the surrounding aqueous solution of substances.

It must, however, be remarked that there still remains very much that is hypothetical in the ideas of Fischer and Lepeschkin on the submicroscopic structure of the living material. The complex chemical compounds, which, in the opinion of Fischer and Lepeschkin, form the live substance, are, to all appearances, complex coacervates, the mechanism of whose formation and whose inner physico-chemical structure have for a long time already been the subject of lively discussion. On the basis of the available facts it may be supposed that the properties of the protoplasm as a phase depend precisely on its coacervate nature. This is a view to which many cytologists, physiologists and biochemists tend more and more (Oparin, 1941, 1957; Guilliermond, 1941; Makarov, 1948a, etc.).

According to the theory developed by Lepeschkin, Nasonov and Fischer, and also certain other workers, the greater or lesser permeability of cells for any substance is to be explained not by the greater or lesser penetration of the substance through the cell membrane, but by the difference in the solubility of the substance in the protoplasm and the surrounding aqueous medium and by the adsorptive or chemical binding by the cell colloids of the matter penetrating the cell. If we use the one term "sorption" to cover these three phenomena (solubility, adsorption and chemical interaction), then the theory of cell permeability developed by these authors may be called the "sorptive theory of cell permeability".

It must be remarked here that in this case the term "permeability" does not properly reflect the nature of the property of cells under consideration. The term is used by the proponents of the membrane theory to denote the ability of a definite obstruction, a barrier separating the contents of the cell from the surrounding medium, to admit through itself certain substances, while the proponents of the sorptive theory, when they use this term, have in mind the ability of the whole contents of the cell to absorb certain substances from the surrounding medium and to excrete them (no reference is intended here to the secretory activity of cells, which must be considered separately). In view of this the phenomenon which has been given the name "permeability of cells" would better have been called the "sorptive activity of cells". But the term "permeability" is deeply rooted in physiology and has become generally used among the majority of biologists, so that it is expedient to keep the term, giving it, however, a different sense.

At present a vast amount of evidence has been accumulated which con-

firms the correctness of the sorptional theory and cannot in any way be fitted into the framework of the membrane conception. In this book, after a critical review of certain membrane theories, part of these facts will be set out systematically and an attempt will be made to determine the part played in the phenomena of cell permeability of each of the above mentioned factors of sorption individually.

## CHAPTER I

# The Membrane Theory of Cell Permeability

### *1. The Fundamental Propositions of the Classical Membrane Theory*

The membrane theory of cell permeability arose, as is well known, at the end of the last century. Its origin is to be found in the works of Pfeffer (1877) and de Vries (1884, 1885, 1888). These authors established that in solutions of certain substances plant cells with a central vacuole behave like osmometers: their volume changes in inverse proportion to the osmotic pressure of the medium. The role of the contents of the cell-osmometer was ascribed by these authors to the contents of the vacuole. It was not clear, however, whether the whole thickness of the protoplasm or only some part of it played the part of the semi-permeable membrane in the cell. Pfeffer supposed that the semi-permeable membrane was localised on the surface of the protoplast and behaved like a Traube sedimentation membrane, being easily permeable for water, but only with difficulty or not at all for the molecules of water-soluble substances.

The idea that there must be on the surface of the protoplasm a layer different in constitution and structure from the rest of the mass of the protoplasm had appeared much earlier. Thus Schultze (1863) and Kühne (1864) asserted that the protoplasm of plant and animal cells is an aqueous solution of organic (principally protein) and mineral substances. At that time it was already known that the protoplasm or fragments of it are immiscible with the surrounding aqueous solution. It was to explain this fact that the supposition was made of the presence on the surface of the protoplasm of cells of a special film, which is not water-soluble and therefore prevents the protoplasm from dissolving. In Kühne's opinion, this film consists of coagulated proteins. In contrast to Kühne, Schultze thought that it was a condensed layer of the protoplasm itself.

The ideas of modern supporters of the membrane theory about the physico-chemical structure and properties of live cells differ little from those of Kühne and Schultze; the only significant change has been in the view taken of the mechanism of formation, the composition and properties of the boundary layer.

The conception of the protoplasm as a simple aqueous solution of substances inevitably leads to the assumption of the presence at the surface of any live cell of a special barrier separating one aqueous solution of substances

(the contents of the cell) from another, also aqueous, solution, the habitat of the cell. Thus, Rubinshtein (1949a) writes: "If the protoplasm is an aqueous colloidal system, then only the presence of a semi-permeable surface membrane makes it possible to explain the features of the distribution of substances between the cell and the external medium and all the characteristic phenomena of cell permeability" (p. 130). Almost identical expressions are used by many other workers as well in postulating the presence of a semi-permeable cellular membrane (Gellhorn, 1929; Osterhout, 1933, 1940; Höber, 1945; Heilbrunn, 1952, etc.).

After the work of Pfeffer, de Vries and Overton (1895–1902), a wide circle of biologists showed a considerably increased interest in the problem of cell permeability, because these authors (especially Pfeffer) pointed out the great importance of the part that the semi-permeable membrane must play in metabolism: it as it were regulates the entrance of substances into the cell and their excretion from the cell and thereby influences the course of cellular metabolism.

From the point of view of the membrane theory, the so called "osmotic behaviour" of animal and plant cells can serve as a good indicator for deciding whether some substance does or does not penetrate the cell. It is considered that if in a solution of some substance a plant cell decreases in volume (plasmolysis) and if during the whole time the cell remains in this solution there is no increase in volume again (deplasmolysis), this may indicate that the semi-permeable cell membrane is completely impermeable for the given substance and that the osmotic pressure of this solution is greater than the osmotic pressure of the contents of the cell under normal conditions. If, however, in a solution of some other substance there occurs first plasmolysis, but then with the passage of time the reverse development (deplasmolysis), this indicates that the membrane-osmometer is permeable for the given substance and that the osmotic pressure of this solution is higher than that of the contents of the cell normally is. In this case the speed of deplasmolysis is also used to judge the speed of diffusion of substances through the boundary layer of the cell: the quicker deplasmolysis sets in, the quicker the given substance penetrates the semi-permeable membrane. This "osmotic behaviour" of cells is the basis of the very widespread so called "osmotic method" of studying cell permeability and the several variants of this method (plasmatic, plasmometric, etc.). Furthermore, the "osmotic behaviour" of cells has up to now been considered the fundamental argument for the membrane conception proving the presence on the surface of the cell of a semi-permeable membrane (Hill, 1935; Höber, 1945; Krogh, 1946; Rubinshtein, 1949b; Vorontsov, 1949; Heilbrunn, 1952, etc.).

Since Pfeffer's time these methods have been used to study the penetration into plant and animal cells of a vast number of the most varied chemical compounds. An especially large number of "osmotic experiments" was performed by Overton, who transferred the idea of plant cells as osmometers to

animal cells as well. He investigated more than 500 substances. On the basis of his experiments Overton (1895, 1896, 1902a) evolved a series of substances, placing them in the order of the speed with which they penetrated cells, and formulated the so called laws of permeability, which established the relation between the speed with which water-soluble substances passed through the cell membrane and their chemical structure. Overton's series and laws are still accepted by proponents of the membrane theory at the present time.

*Overton's series is as follows*

- (1) Very quick penetration is achieved by: hydrocarbons (saturated, unsaturated and cyclic) and their halogen derivatives, monohydric alcohols, aldehydes, ketones, nitrites, ethers, many organic acids and bases.
- (2) Quick penetration: dihydric alcohols and amides of monobasic acids.
- (3) Less quick penetration: glycerol, urea and thiourea, etc.
- (4) Slow penetration: tetrahydric alcohols.
- (5) Very slow penetration or none at all: hexahydric alcohols, sugars (pentose, hexose, disaccharides, etc.), a wide range of amino acids, many neutral salts of organic acids, strong mineral acids, and also the salts of strong mineral acids and alkalis.

According to Overton, the ability of a substance to penetrate the cell increases with the length of the hydrocarbon chain in the molecule and the number of methyl, ethyl and phenyl groups. The degree of penetration is considerably reduced with the introduction into the molecule of hydroxyl, carboxyl or amino groups.

Overton's conclusions, reached on the basis of his "osmotic experiments", are astonishingly paradoxical. In fact, it is hard to agree with such an assertion as that carbohydrates, amino acids and salts, which play an exceptionally important part in the life of the cell, are not able to penetrate it. After Overton's work, a host of experiments on a great variety of subjects was performed to test the theory; the results of these osmotic experiments confirm Overton's conclusions.

Directly contradictory conclusions were, however, reached by other workers studying the permeability of plant and animal cells by other methods (indirect chemical analysis of the contents of cells, the method of "labelled atoms" etc.).

The results obtained by these scientists are of great biological interest. They demonstrate irrefutably the permeability of live cells for carbohydrates, amino acids, salts and other substances which (to judge by the "osmotic experiments") are unable to pass through the cell membrane. These data will be given in detail below.

Further, it will be shown that the "osmotic" method is not valid for the study of cell permeability, because it must now be taken as firmly established

that animal and non-vacuolar plant cells cannot be considered as osmometers: their behaviour in solutions of various substances does not obey the laws of osmosis. Therefore conclusions about the ability or inability of some substance to permeate a cell, if made on the basis of this method, must be considered incompetent.

## *2. The Chemical Composition and Structure of the Cell Membrane*

The above mentioned series of permeating substances gave grounds for certain conclusions about the chemical composition and physico-chemical structure of the protoplasmic membrane, whose properties must be responsible for the varying rates of penetration into the cell of different substances.

The "protoplasmic membrane" is a physiological, not a morphological, concept. The supporters of the membrane theory always draw attention to this aspect of the matter to emphasise that the semi-permeable cell membrane has nothing in common with those envelopes that are so clearly visible in some cases (Overton, 1902a; Höber, 1926, 1945; Harvey and Danielli, 1939, etc.). Their semi-permeable membrane is a very thin film, invisible even under the highest magnification of a light microscope. For example, the protoplasts of plasmolysed plant cells, the unfertilised eggs of certain sea animals, leucocytes and certain other cells have on their surface such a protoplasmic membrane, which is in direct contact with the medium on one side and the contents of the cell on the other. Similar semi-permeable membranes, in the opinion of many workers, also surround the various visible structural forms in the cell, such as granules, vacuoles, etc.

There are no direct proofs of the existence of such a membrane on the surface of live cells: their presence and structure are inferred from indirect physico-chemical data and electron-microscopic observations on fixed cells. "All our conclusions about the existence and nature of this semi-permeable envelope, its structure and chemical composition", writes Rubinshtein (1947), "are based on the investigation of the passage through it of various soluble substances and on comparison with artificial membranes with similar properties" (p. 106).

Some authors, however, consider that it is impossible to give a concrete picture of the structure of the semi-permeable membrane. Thus, for example, Lebedinskii (1939) says: "...the membrane should be thought of primarily as a combination of biological processes which allow the work to be done" (p. 116). For most investigators, as Kan (1939) rightly observes, "the membrane is of purely conventional significance, a purely symbolical term" (p. 110).

On the basis of the abundance of experimental material on the permeability of plant and animal cells obtained by the osmotic method, Overton (1899) developed the lipoid theory of cell permeability, based on an idea of Quincke

(1898) that any live cell must be covered by an external film of fat or oil to prevent the protoplasm mixing with the water of the surrounding medium. According to Overton, the plasma membrane is a film of fat-like substances—lipoids.

The rate of permeation of a solute into and out of a cell is determined by its coefficient of distribution between the lipoid and water phases. The greater this coefficient, the better the substance should penetrate the cell. In proof of this, Overton adduced data about the distribution of the substances he studied between water and a mixture of lecithin and cholesterol dissolved in organic fluids (chloroform, xylol and others). In many cases a parallelism was observed between the rate of permeation of substances into a cell and the magnitude of the coefficient of their distribution between this mixture and water.

Overton's lipoid theory formed the basis of a series of large scale biological generalisations; but it was soon abandoned because of its inability to explain a number of indisputable facts, such as: the permeability of cells for water, urea and many other substances which penetrate into cells very quickly but are completely insoluble in lipoids. None the less, substantial elements of the lipoid theory remain in many contemporary theories of cell permeability.

Nathanson (1904a, 1904b), in attempting to improve Overton's theory, put forward the suggestion that the plasma membrane is mosaic in structure. He suggested that there is cholesterol on the surface of the cell between the live particles of the protoplasm. This should explain the permeation into cells of substances insoluble in lipoids as well as soluble substances. Nathanson's theory had no experimental foundation, but the idea of two kinds of permeation of substances into cells thereafter occupied a fundamental position in the development of theories of the structure of the semi-permeable membrane.

In a series of articles, Ruhland (1908–1913) developed his well known theory of the ultrafilter. In his opinion, the speed of penetration of a substance into cells depends on its molecular volume. According to this theory, the semi-permeable membrane works on a sieve principle. It has, as it were, a mesh of definite magnitude, and those molecules whose diameter exceeds that of the holes do not penetrate into the cell. In proof of this he adduced the fact that a live cell will take up both dyes soluble in lipoids and those that are not, while those dyes that do not penetrate into the cell form dispersed colloidal solutions.

Subsequently, Ruhland and Hoffmann (1925) apparently succeeded in proving experimentally the applicability of the ultrafilter theory to the permeability of the sulphur bacteria *Beggiatoa mirabilis* for a large number of organic compounds that form real solutions in water.

The data given by these authors are shown in Table 1: they show that for a large number of substances there is a parallelism between the fall in magni-

tude of the threshold plasmolytic concentration (which served as the basis for their determination of the permeation of substances into the cell) and the increase in magnitude of the molecular refraction ( $MR_D$ ) of these substances.

TABLE 1. THE PERMEABILITY OF *Beggiatoa mirabilis* FOR NON-ELECTROLYTES  
(after Ruhland and Hoffmann, 1925)

Substance	Threshold plasmolytic concentration	$MR_D$	Distribution coefficient between: ether-water
Urea	0.35	16.67	0.0005
Ethylene glycol	0.09	14.40	0.0068
Methylurea	0.01	18.47	0.0012
Thiourea	0.075	19.59	0.0063
Glycerol	0.009	20.63	0.0011
Ethylurethane	0.015	21.01	0.6370
Lactamide	0.007	21.13	0.0018
Malonamide	0.007	22.92	0.0003
Dimethylurea	0.005	23.43	0.0116
Butyramide	0.00125	24.11	0.0580
Erythritol	0.001	26.77	0.0001
Succinamide	0.0015	27.54	0.0002
Arabinose	0.0008	31.40	0.0001
Diethylurea	0.003	32.66	0.0185
Glucose	0.00055	37.54	0.0001
Mannitol	0.00055	39.06	0.0001
Sucrose	0.00020	70.35	0.0001

In their experiments they did not find this coincidence with the magnitude of the distribution coefficient of substances between ether and water, which contradicts Overton's theory.

In support of the sieve theory a multitude of model experiments were performed with artificial membranes made of collodion, gelatine and other materials. Many methods were devised for the preparation of membranes which could readily admit through themselves only molecules under a certain size (Brown, 1915; Collander, 1924, 1926; Michaelis *et al.*, 1925-1927; Fujita, 1926; Höber and Hoffmann, 1928, and many others).

The sieve theory could not, however, explain the familiar fact that the permeability of many plant and animal cells for substances of a homologous series increases with the increase in the chain of the number of carbon atoms. For plant cells this proposition was confirmed by Collander and Bärlund (1933) by the direct chemical analysis of the cell sap of the algae, *Characeae*.

These authors found that for a whole series of substances there is a correspondence between the speed of their penetration into the cell vacuole and the magnitude of the coefficient of distribution in the system: olive oil and

water, while for other substances there is no such correspondence. Their speed of penetration increases with the decrease in the volume of the molecule. On the basis of these data the authors combined the lipoid theory and the molecular sieve theory in one. According to this lipoid-filtration theory, the permeation of substances insoluble in lipoids is regulated by the size of the pores in the membrane, while that of substances soluble in lipoids is governed by the coefficient of distribution of the substances between the non-aqueous and the aqueous phases. This theory has, at present, many adherents (Höber, 1936a, 1945; Wilbrandt, 1938; Rubinshtein, 1947, etc.).

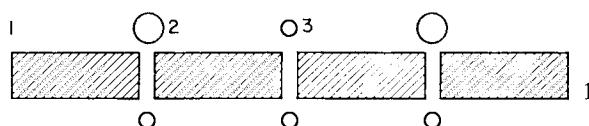


FIG. 1. Diagram showing the structure of the semi-permeable cell membrane  
(from Seifriz, 1936).

1—membrane, 2—sugar molecules, 3—water molecules.

In Fig. 1 is shown the scheme of such a membrane, explaining the mechanism of the penetration of water molecules into the cell and the impermeability of cells for sugar molecules, because this is insoluble in lipoids and the diameter of its molecules is greater than the diameter of the openings in the membrane.

Many investigators have tried more or less exactly to represent the chemical composition and molecular structure of the protoplasmic membrane. Thus, according to Parpart and Dziedzian (1940), the plasma membrane consists basically of lipoids and proteins in the ratio 1 : 1.7. According to their data, the lipoids are represented principally by phospholipids, which are, as is well known, hydrophilic colloids. There is also, in their opinion, in the composition of the membrane a hydrophobic substance—cholesterol, which, in conjunction with the proteins and lipoids, makes the membrane insoluble in water. The conjunction of these substances in definite proportions also explains the properties of the membrane both as a solvent and as a sieve. This conjunction of substances and also the ratio in the membrane of saturated and unsaturated acids, as it were, explains certain conspicuous differences in permeability of cells of different types of animals and plants (Höber, 1936a, 1936b).

After the work of Langmuir, Adam and others the protoplasmic membrane began to be identified with monomolecular and bimolecular films, whose molecules have a definite orientation in relation to one another. In the estimation of Danielli (1935, 1936), and also Harvey and Danielli (1939), the protoplasmic membrane should consist of two oriented layers of lipid molecules, whose hydrophilic ends point in opposite directions (to the inside and outside of the cell). The molecules of the oriented layers of the mem-

brane are on the surface of the cell in the form of a palisade, as depicted in Fig. 2. Because the surface tension of the protoplasm is very low (for example, the eggs of sea urchins about 0·1–0·2 dyne/cm), it is supposed that the membrane is covered on both sides with a layer of globular proteins (see Fig. 2), which greatly reduce the surface tension of the cell.

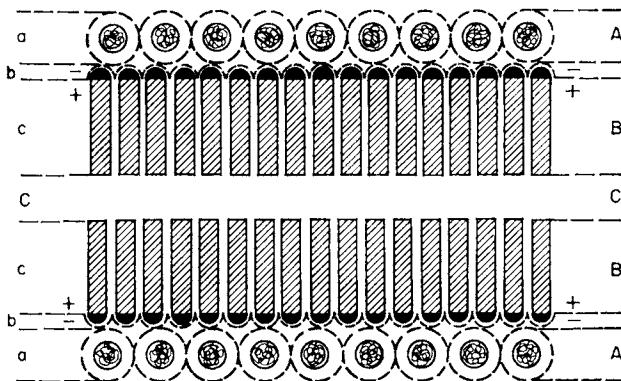


FIG. 2. Diagram showing the structure of the semi-permeable membrane according to Danielli and Harvey (from Höber, 1945).

A—layer of globular proteins, B—layer of oriented lipid molecules, C—unoriented layer of lipid molecules; a—hydrated protein molecules, b—ionised ends of lipid molecules, c—unionised ends of lipid molecules, a + b hydrophilic zone, C + c lipophilic zone.

At present, a large number of schemes have been suggested for the structure of the cell membrane, but they all differ only in inessential details from the scheme shown in Fig. 2 (Davson and Danielli, 1943; Holtfreter, 1948; Rubinststein, 1949b; Danielli, 1952a, b, c; Booij and Bungenberg de Jong, 1956, and others).

Bungenberg de Jong and Bonner (1935) produced the hypothesis of the molecular structure of the membrane as an autocomplex coacervate, consisting of molecules of phosphatides condensed with molecules of cholesterol (see Fig. 3). In the opinion of these authors, the membrane also consists of two layers of molecules, whose hydrophilic ends are turned, not outward, as supposed in Harvey and Danielli's scheme, but towards one another.

A number of authors have tried to reach a conclusion also about the thickness of the membrane. Thus, Gorter and Grendel (1925) determined chemically the amount of lipoids in erythrocytes and calculated that, to cover the whole surface of the erythrocyte, there was enough of them for only one layer. According to the analyses of Dziemian (1939, 1942), there is on the average in the erythrocytes of various animals enough lipoids to form an unbroken layer, 30 Å thick, covering the whole external surface of the cell.

In the opinion of Fricke, Parker and Ponder (1939), the plasma membrane is about 100 Å thick, since it consists, so they suppose, of proteins as well as lipoids. According to Schmitt, Bear and Ponder (1938), who observed erythrocytes in polarised light, the membrane is 200 Å thick, while, after the extraction of the lipoids, it falls to 120 Å thick. Fricke (1925), taking cells to be covered by a non-conducting layer, measured the electrical conductivity of a suspension of erythrocytes at various frequencies and calculated the "capacity of the membrane". According to these data, assuming that the membrane consists of fat-like substances with a dielectric constant of 3, he determined the thickness of the membrane as 33 Å. According to Danielli (1936), the dielectric constant of the cell lipoids is 5, on which basis he suggests that the membrane is about twice as thick and speaks of its being made up of two layers of lipoid molecules.

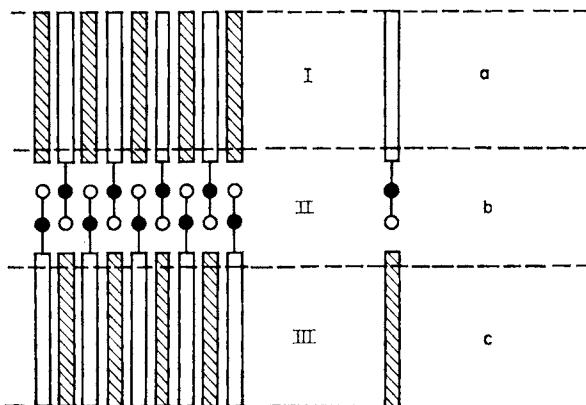


FIG. 3. Diagram showing the structure of the semi-permeable membrane according to Bungenberg de Jong and Bonner (1935).

I and III—lipophilic zones, II—hydrophilic zone; a—hydrophilic part of phosphatide molecule, b—hydrophilic part of phosphatide molecule, c—sensitised cholesterol molecule.

The "capacity of the membrane" of muscle and nerve fibres, and also of other cells, varies within the limits 1 to 10  $\mu\text{F}/\text{cm}^2$  (Hodgkin, 1951, 1958; Katz, 1956; Eccles, 1957). According to the data of Curtis and Cole (1938), the capacity of the membrane of the giant axon squid is 1  $\mu\text{F}/\text{cm}^2$ . This corresponds to a bimolecular layer of lipoids 50 Å thick with a dielectric constant for the lipoids about 5.

It seems astonishing that contemporary electron-microscopic investigations of various cells and nerve and muscle fibres lead to a similar conclusion about the structure of the protoplasmic membrane. Thus, the membrane of the nerve cells of the frog, according to electron-microscopic data, is 50–100 Å thick (Geren and Schmitt, 1954; Robertson, 1957). The membrane of the

giant nerve fibre of the squid is about the same (Frankenhaeuser and Hodgkin, 1956).

According to contemporary electron-microscopic data, the most diverse cells, fixed with osmic acid or permanganate, have double membranes covering the whole outside of the cell. Mitochondria and certain other cell organelles have on their surface a membrane of a similar sort. Further, mitochondria are divided by similar membranes into many chambers. Certain cell organelles, for example, Golgi's apparatus, consist entirely of such a membrane. And the protoplasm itself, according to these data, contains a great number of closely adjoining plates (membranes). All these membranes are 150–300 Å thick on the average. The thickness of each of the two osmophilic layers of the double membrane is 35–70 Å, and that of the more transparent layer between them 40–80 Å (Sjöstrand, 1955a, 1955b; Claude, 1955; Palade, 1955; Gatenby, 1954; Karpas, 1959; Oberling, 1959).

### *3. Hypotheses of the Formation of Semi-permeable Membranes on the Surface of the Protoplasm*

According to contemporary ideas, the mechanism of the formation of the semi-permeable cell membranes is similar to that of the monomolecular and bimolecular adsorptional films of Langmuir. The identification of Langmuir's film with the semi-permeable cell membranes rests on the conviction that the protoplasm is a complex mixture of colloids and crystalloids in water, including proteins and lipoids (see Fig. 4). At the boundary of the protoplasm and the surrounding water, therefore, there must be concentrated the surface active substances and, primarily, the lipoids (Gellhorn, 1929; Rubinshtein, 1939, 1947, 1949b; Kizel', 1940; Schmidt, 1941, and others).

However, these arguments cannot be considered convincing. Lepeschkin (1924), Nasonov and Aleksandrov (1940) and Nasonov (1949a) pointed out that such a mechanism of formation of the semi-permeable membranes cannot be applicable to cells, because it contradicts the initial assumption of the membrane theory, according to which the protoplasm is a mixture of various substances in water. Under these circumstances, there is no dividing surface between the protoplasm and the surrounding water where the lipoids and the other substances could be adsorbed: there is only the surface separating the colloidal particles of the protoplasm and the water.

According to Heilbrunn (1928, 1930, 1952, 1956), when the protoplasm comes into contact with the medium, there is quickly formed on its surface an envelope of coagulated proteins which prevents the contents of the cell from being dissolved. In the opinion of many authors, it is also the basis for the formation of the actual semi-permeable membrane.

Heilbrunn observed the formation of this envelope of coagulated proteins chiefly in the eggs of echinoderms. In his opinion, there lies at the basis of the mechanism of the formation of this envelope a fermentation process similar

to the coagulation of the blood, in which calcium ions are also of great importance ("surface precipitation reaction"). Similar envelopes, as he supposes, are also formed within the cell, for example, around the vacuoles.

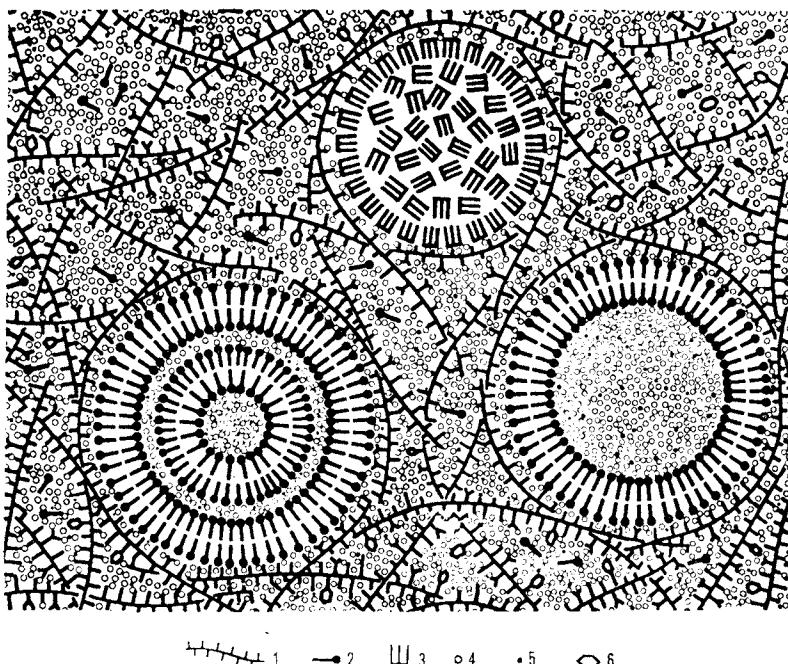


FIG. 4. The submicroscopic structure of the protoplasm (from Schmidt, 1941).

1—protein molecules, 2—lipid molecules, 3—triglyceride, 4—water molecules, 5—ions, 6—other substances; on the right—a vacuole with aqueous contents, surrounded by a lipid membrane; on the left—a lipid drop; above—a fat drop.

However, Lepeschkin (1937, 1939) did not observe this phenomenon. In a number of cases, the protoplasm, when isolated from the cell, really does not mix with the medium, but without the formation of an envelope. Lepeschkin (1926a) dispersed *Bryopsis* cells in sea water; thereupon many drops of protoplasm formed which did not mix with the water. On dilution of the sea water the protoplasm droplets swelled enormously, and then vacuoles appeared in them. When the droplets were returned to the sea water, the vacuoles disappeared without trace, nor were there any traces of Heilbrunn's envelopes around these vacuoles.

There is another circumstance that militates against this opinion that Heilbrunn's envelopes can be the basis for the formation of actual semi-permeable membranes, as asserted by Rubinshtein (1947, 1949a) and others: according

to all the experimental data, the surface of the cell is a liquid (Seifriz, 1936, 1941; Makarov, 1948a, and others), while Heilbrunn's envelopes are solid formations. When cells are dispersed, a vast number of protoplasmic droplets can be formed which are immiscible with an aqueous solution. According to the membrane theory, this means that a lipoid membrane has formed on the surface of each droplet. In this case, however, the surface of the protoplasmic droplets can be increased a thousand times in comparison with the surface of the unbroken cell (Lepeschkin, 1924). Further, when certain agents act on a variety of cells, a vast number of vacuoles may be formed in the protoplasm of the latter, giving it a frothy appearance (Lepeschkin, 1924, 1937; Heilbrunn, 1928; Nasonov and Aleksandrov, 1940). If all these protoplasmic droplets and vacuoles really had lipoid films around them, as required by the membrane theory, then the quantity of lipoids contained in the cell would be inadequate for the formation of so large an area of membrane in it. Further, Seifriz (1941) adduces against this way of formation of a lipoid membrane the argument that many of the simplest pseudopods can be greatly elongated, which should lead to a breakdown of the lipoid film, and here again there will not be sufficient material for the re-establishment of the membrane in the elongated pseudopods.

Consequently, the possibility of the formation on the surface of cells of lipoid membranes of the type of Langmuir's adsorptive layers is altogether doubtful. However, if such a membrane actually does exist on the surface of the protoplasm, as indicated by the indirect data and electron-microscopic investigations, nonetheless it cannot play the part frequently ascribed to it. Factual data in support of this will be cited below.

#### *4. Membrane Theories of the Mechanism of the Penetration of Substances into Cells*

The classical membrane theory of cell permeability explains the passage of a substance from the surrounding medium into a cell or in the reverse direction as a process of diffusion through a semipermeable membrane, which is seen as a passive structure. Therefore the quantitative characteristics of cell permeability are derived from Fick's law, which is applicable to the free diffusion of solutes in a homogeneous medium. This law, as is well known, may be written in the following form:

$$dQ = - DA \frac{dc}{dx} dt,$$

where  $dQ$  is the quantity of solute diffusing in time  $dt$ ;  $A$  the area through which diffusion takes place;  $dc/dx$  the concentration gradient,  $dx$  being the distance in the direction of diffusion in which the solute concentration falls by the amount  $dc$ .  $D$  is a constant called the diffusion coefficient. The mag-

nitude of this coefficient also gives the quantitative character of the diffusion properties of any substance. The coefficient  $D$  will be given by the amount of the substance diffusing in unit time through unit area with unit concentration

$$\text{gradient} - \frac{dc}{dx} = 1.$$

In its application to the permeability of cells for solutes Fick's law is changed as follows: the concentration gradient is replaced by the difference in concentration of the permeating substance on either side of the membrane ( $C_e - C_i$ ), while the diffusion coefficient  $D$  is replaced by the permeability coefficient  $P$ , which, however, includes not only the quantitative characteristics of the diffusion properties of the substance but also, in a hidden form, the thickness of the membrane. Thus, the permeability formula, analogous to Fick's law, looks as follows:

$$dQ = PA(C_e - C_i) dt.$$

In this equation  $dQ$  is the amount of the substance penetrating the cell in time  $dt$  through an area of the membrane  $A$ , the difference in concentration inside and outside the cell being  $C_e - C_i$  (Collander and Bärlund, 1933; Jacobs, 1935; Rubinshtein, 1947, and others).

The same formula is used for the transfer of water into and out of the cell, except that the concentration difference is replaced by the difference in the osmotic pressure of the medium and the contents of the cell. The speed of penetration of water through the membrane is in this case determined by the speed of change of volume of the cell in hypotonic and hypertonic solutions of substances which either fail to penetrate the cell or do so only very slowly (Stewart, 1931a; Jacobs and Stewart, 1932; Stewart and Jacobs, 1932a, b, 1936; Lucké, 1940; Rubinshtein, 1947, and others).

The magnitude of the permeability constant  $P$  for many substances, electrolytes and non-electrolytes, in a wide variety of plant and animal cells has been determined by many investigators. Thus Collander and Bärlund (1933) studied the speed of penetration of a large number of electrolytes into the vacuole of the cells of the internodes of the algae *Characeae*. They placed the cells in solutions of the substances under investigation and determined the time in which the concentration of these substances in the cell sap (the sap was withdrawn from the cells by pipette) reached half their concentration in the external liquid. For comparison, the authors also determined the speed of penetration of substances into previously killed cells and calculated the time taken to reach half concentration for a column of the liquid of the same size as the cells of the algae. In that case, when the volume of the cell in the course of the experiment does not change, the initial concentration of the permeating substance in the cell is equal to zero, while its concentration in the medium during the experiment remains practically unchanged (in view of the large quantity of the surrounding solution), the constant of perme-

ability  $P$  being then determined by the simple equation:

$$P = \frac{V}{At} \ln \frac{C_e}{C_e - C_i},$$

where  $V$  is the volume of the cell, and  $C_e$  and  $C_i$  the concentration of the substance in the medium and in the cell sap. From this formula it is easy to calculate the time  $t_x$  needed to bring the concentration of the substance in the cell (or in a column of liquid of the same magnitude) to half its concentration in the medium, the formula becoming the following:

$$t_x = \frac{V}{AP} \ln 2.$$

Collander and Bärlund obtained the values of  $t_x$  indicated in Table 2.

TABLE 2. THE SPEED OF PENETRATION OF NON-ELECTROLYTES INTO LIVE AND DEAD CELLS OF THE ALGAE *Characeae*  
(after Collander and Bärlund, 1933)

Substance	Time needed for the concentration in the cell sap to reach half the external concentration ( $t_x$ , min)		
	Found directly by experiment for		Calculated for a column of water equal in volume to the cells
	Live cells	Dead cells	
Methyl alcohol	1·3	0·8	0·27
Urea	320·0	0·9	0·37
Acetamide	24·0	1·2	0·38
Glycerol	1700·0	1·9	0·49
Trimethyl citrate	5·5	2·2	0·67
Sucrose	750,000·0	4·1	0·92

From this table it follows that the time needed to reach half concentration in the cell sap of dead cells is determined by the magnitude of the diffusion coefficient, just as in water for a column of liquid of the same size. The only difference is that in the first case, this coefficient is 3–4 times greater for all substances by comparison with the coefficient of free diffusion; for live cells, the order of magnitude of the time of reaching half concentration in the sap (and, consequently, also of the coefficient  $P$ ) is completely different: for methyl alcohol and trimethyl citrate the time of reaching half concentration in the sap is little greater than in dead cells and a column of liquid, while for urea and glycerol the time is one to three thousand times greater and for sucrose almost a million times. This means that the change in the permeability coefficient  $P$  in the series of substances mentioned is completely different in nature from its reduction in comparison with the coefficient of free

diffusion and of the diffusion of substances into dead cells. On the basis of the work cited the authors came to the conclusion that all this depends on the character of the semi-permeable membrane, which, they suggest, works both as a molecular sieve and as a lipoid membrane. They studied the penetration of 45 substances into the vacuole of the cells of *Chara ceratophylla*. The penetration of some of these is determined, in their opinion, by the coefficient of distribution between the fat-like substances of the membrane and the water. The basis for this assumption was that they found a certain parallelism between the magnitude of  $P$  and the value of the coefficient of distribution between olive oil and water. The other part of the substances, which dissolve only with difficulty in oil and have a molecular refraction about 15, permeates through the pores of the membrane.

In the opinion of Collander and Bärlund, the principal resistance to the diffusion of substances into the vacuole is offered by the protoplasmic membrane and to a lesser degree by the tonoplast (vacuole envelope), while the protoplasm itself as a medium for the diffusion of substances is similar to the surrounding water.

In the opinion of these authors, sugars (arabinose, glucose, fructose, sucrose and maltose) almost completely fail to penetrate the vacuole. However, it does not at all follow from their data that these substances do not penetrate the protoplast. Further, the method of comparing the speed of penetration of substances into the vacuole by the time to reach half the external concentration cannot be considered faultless, because for certain substances (especially sugars) in the concentrations studied it is possible that half the external concentration was actually the equilibrium diffusion concentration in the sap. There is a certain amount of foundation for such a supposition. Thus, the authors themselves indicate that the equilibrium concentration in the cell sap for methyl alcohol reaches 70 per cent of the external concentration. For certain other substances their equilibrium concentration in the vacuole is also significantly lower than their concentration in the surrounding medium.

According to the ideas of Osterhout (1933, 1940, 1955), the protoplasm of plant cells, which is an aqueous medium, is separated from the surrounding medium and the aqueous solution of the vacuole by liquid non-aqueous phases—the protoplasmic membrane and the tonoplast. The varying speed of penetration of substances through these non-aqueous phases is a result of their varying solubility in the membrane or their chemical interaction with the molecules of these non-aqueous phases; inorganic substances, as a rule, pass through these membranes only in the undissociated form. This conception of the properties of the cell membrane and tonoplast contradicts much experimental material, including Osterhout's own data. He showed (Osterhout, 1950) that the speed of diffusion of water through the cell membrane and the tonoplast of *Nitella* is more than 18 times greater than that of ethyl alcohol. This contradicts his conception, as the author himself says.

From the point of view of the membrane theory, the speed of penetration into the cell of substances whose molecules have sharply defined electrical polarity depends on the size of the molecule, because these substances are not soluble in lipoids and can enter the cell only through the pores of the membrane. The speed of penetration into the cell of non-polar substances is basically determined by their varying solubility in lipoids. The greater the solubility of such a substance in lipoids, the faster its penetration into the cell; consequently, the coefficient  $P$  will be the greater, the greater the solubility of the given substance in fat-like substances. The volume of the molecule in this case does not influence the speed of penetration of the substance into the cell, while in certain cases increase in the size of the molecule even enables it to pass more quickly through the membrane (Höber, 1936a, 1945; Rubinshtain, 1947; Collander, 1947).

However, Nirenstein (1920) found that the intensity of colouration of infusoria by vital dyes coincides better with the solubility of these dyes in a mixture of fat and fatty acids than with their solubility in lipoids.

Besides this, the solubility of substances in organic liquids is not the only physical property which runs parallel to their speed of diffusion into the cell. Traube (1904a, 1904b, 1908, 1910, 1913) established that the more a substance of the homologous series is adsorbed at the water/air barrier, the quicker it penetrates the cell. According to Traube, the permeation of a substance into the cell and also the narcotic strength depends on its ability to be adsorbed in the membrane. The better the substance is adsorbed by the membrane, the more chance it has of penetrating the cell.

Traube's permeability theory had, it appeared, certain advantages over Overton's theory, because Loewe (1912, 1922) showed that the latter was in fact dealing not with the solubility of substances in lipoids, but with the adsorption on lipoids, which, in the mixtures with chloroform and xylol which Overton used in his experiments, form not true solutions but colloidal dispersion systems. But Krebs and Nachmansohn (1927) observed that the penetration of vital dyes into paramecia agrees well both with the solubility of these substances in lipoids and with their adsorption on kaolin. Further, Fleischmann (1928) demonstrated that many substances, including also those that are not surface active at the water/air boundary, penetrate plant cells (and erythrocytes) better the more strongly they are adsorbed on carbon.

It should, however, be borne in mind that the solubility of substances in lipoids or other organic substances, and likewise their adsorption, cannot be used as proof that these physical phenomena can affect the entrance of substances into the cell only in the case when they take place on the surface of the cell and not in the whole thickness of the protoplasm.

At present, a theory that has gained wide recognition is the "activated diffusion" theory, put forward by Danielli. In this theory the cell membrane is basically a lipoid film completely without pores, as shown in Fig. 2. The penetration into the cell of a substance is thought of as a passage in the

interstices between the molecules of the lipoid film, independently of whether the given substance is soluble in lipoids or not. The molecules of the permeating substance "punch" their way through, pushing apart the lipoid molecules in the membrane (Harvey and Danielli, 1939; Medvedev, 1939; Davson and Danielli, 1943; Höber, 1945; Rubinshtein, 1949b, and others). It is supposed that the molecules of the dissolved substances strike against the membrane and break up the arrangement of the lipoid particles (form a temporary pore), thus passing into or out of the cell. To be able to do this the molecules of the solute must have a definite kinetic energy to overcome the potential energy barrier of the plasma membrane. In support of these ideas certain results are cited which indicate that the temperature coefficient ( $Q_{10}$ ) for permeability is too high to allow this phenomenon to be regarded as a normal diffusion process (Harvey and Danielli, 1939; Davson and Danielli, 1943; Danielli, 1952a, b).

On the basis of a study of the permeability of colloidal films Michaelis put forward his well-known theory of the permeability of the protoplasmic membrane for ions. In the work of Michaelis and his colleagues (1925–1927), the dried colloidal membranes have a pore diameter such as to allow the passage of glucose molecules. The pores of such a membrane have a negative charge and are therefore impermeable for similarly charged particles—anions. But cations, interchanging with the compensating cations of the negative charges of the pores, can pass through them if the diameter of the penetrating cations is less than that of the pores. Cations with a diameter greater than that of potassium (sodium and lithium ions, bivalent ions, etc.) cannot pass through such a membrane. According to Michaelis, the semi-permeable cell membrane works on a similar principle.

It must be noted that Michaelis' selective ion permeability is a property only of those dried colloidal membranes which, though impermeable for glucose, are yet permeable for urea molecules. It is important to remember this in evaluating the theory of cell permeability for ions put forward by this author. It is now firmly established that a wide variety of animal and plant cells are permeable not only for monosaccharides, but also for disaccharides and other substances with a still greater molecular volume. This is already enough to show that the mechanism of the permeability of dead membranes for ions cannot be the same as that in live cells.

Of course, there is no reason to doubt the selective permeability of dried colloidal films, as established by Michaelis and his colleagues. In recent years there has again been a vigorous interest in the study of the properties of artificial membranes with the object of describing analogies to that which is observed in the study of the permeability of live cells (Hitchcock, 1955; Scatchard, 1955; Sollner, 1955; Sollner *et al.*, 1955; Teorell, 1959a, 1959b).

If a negatively charged membrane is oppositely charged, as was done by Mond and Hoffmann (1928a), by impregnating it with a basic dye—rhodamine B, then it will pass only anions and block cations.

Höber and Hoffmann (1928) prepared first a negatively charged colloidal membrane, which passed only cations at various rates and not anions. They then impregnated certain parts of it with rhodamine B: these parts then passed only anions at various rates, but not cations.

In the opinion of these writers, the membrane of the algae *Valonia* must be constructed in the same way, because they acknowledge the penetration into this cell of both anions and cations. Brooks (1929, 1932, 1933, 1938b) also speaks of the existence of negatively and positively charged pores in the membrane. Different places for the permeation of cations and anions are also postulated by Boyle and Conway (1941) in the case of the membrane of muscle fibres. In their opinion, muscle fibres may be penetrated by anions and cations whose diameter does not exceed that of  $K^+$  and  $NO_3^-$ . These authors calculated the relative diameter of ions by their mobility in water and place them in the order of the speed with which they should penetrate muscle fibres (see Table 3). In this table, sodium, magnesium and phosphate ions are marked as being incapable of permeating muscle fibres.

TABLE 3. THE RELATIVE DIAMETER OF CERTAIN IONS AND THE PERMEABILITY  
OF THE MUSCLE FIBRES OF THE FROG FOR THEM  
(after Boyle and Conway, 1941)

	Cations	Diameter of cations	Anions	Diameter of anions
Penetrate	H	0.20	OH	0.37
	Rb	0.96	Br	0.96
	Cs	1.00	I	0.97
	NH <sub>4</sub>	1.00	Cl	0.98
	K	1.00	NO <sub>3</sub>	1.04
Do not penetrate	Na	1.49	CH <sub>3</sub> COO	1.84
	Li	1.95	SO <sub>4</sub>	1.89
	Ca	2.51	HPO <sub>4</sub>	2.29
	Mg	2.84		

The ultrafilter theory, developed by Michaelis in application to the selective ion permeability of artificial membranes, received considerable supplementation from Teorell (1935, 1935-6) and Meyer and Sievers (1936).

It is well known that Michaelis derived the quantitative characteristics of the mobility of ions through a membrane from the magnitude of the diffusion potential. Thus, if two solutions of one and the same salt in different concentrations are separated by a membrane, there arises a diffusion potential  $E$ , whose magnitude is given by Nernst's formula, according to which:

$$E = \frac{RT}{F} \frac{U_c - U_a}{U_c + U_a} \ln \frac{C_1}{C_2},$$

where  $U_c$  and  $U_a$  are the mobility of a cation and anion in the membrane, and  $C_1$  and  $C_2$  the concentration of the electrolyte on either side of the membrane.  $E$  reaches its maximum value when either  $U_c$  or  $U_a = 0$ . It follows from this formula that the magnitude of the diffusion potential depends on the value of the ratio  $C_1/C_2$  and should not depend on the absolute value of the concentrations  $C_1$  and  $C_2$ . However, this requirement of the theory is not supported by experiment (Michaelis, Ellsworth and Weech, 1927). Michaelis was unable to give a satisfactory explanation of the phenomenon. Teorell, Meyer and Sievers tried to get round the difficulty as follows.

In their view, the cell membrane is a polyvalent colloidal network, which acts as an anion or cation depending on the pH. In the first case the mobile ions in the membrane will be the cations and in the second, the anions. In the meshes of this network there are carboxyl groups or amino groups or both. It is these groups that are responsible for the selective ion permeability of the membrane. If the polyvalent network behaves as an acid, it splits off the hydrogen ions; then the cations will pass through the membrane more easily than the anions. If the network behaves as a base, then such a membrane will be preferentially permeable for anions. In this case, when the numbers of the dissociated carboxyl and amino groups in the membrane are equal, the membrane will not have this property of selective permeability.

Let us suppose that under the conditions of pH the membrane splits off cations  $K$ , whose concentration  $A$  in the membrane will be equal to the concentration of the immobile anions  $R$ . When this membrane comes into contact with the solution of electrolyte, for example in a concentration  $c$ , the conditions for Donnan equilibrium are established. According to Donnan's law, the product of the concentration of the anions and cations in the membrane and the salt solution must be the same. If the concentration of potassium chloride which has penetrated the membrane is  $y$ , then  $y(y + A) = c^2$ , whence

$$y = \sqrt{\left(c^2 + \frac{A^2}{4}\right)} - \frac{A}{2}.$$

The mobile anion concentration in the membrane will be  $y$ , and the mobile cation concentration  $(y + A)$ .

The number of cations  $n_c$  and anions  $n_a$  penetrating the membrane is proportional not only to the mobility of the ions, but also to their concentration in the membrane. Consequently:

$$\frac{n_c}{n_a} = \frac{U_c(y + A)}{U_a \cdot y}.$$

Substituting in this equation the value of  $y$  obtained from Donnan's law, we obtain:

$$\frac{n_c}{n_a} = \frac{U_c[\sqrt{4c^2 + A^2} + A]}{U_a[\sqrt{4c^2 + A^2} - A]}.$$

Meyer and Sievers call  $n_c$  and  $n_a$  the transfer numbers. The value of the ratio of the transfer numbers ( $n_c/n_a$ ) will be a quantitative measure of the selective permeability of the membrane for ions. From the formula deduced it can be seen that the greater  $A$  is in comparison with  $C$ , the greater will be the ratio of the transfer numbers and the more markedly selective the permeability of the membrane to cations. Vice versa, the greater  $C$  is in comparison with  $A$  (the number of dissociated groups in the membrane), the less marked will this selectivity be. Therefore Meyer and Sievers call  $A$  the "selectivity constant".

These authors found satisfactory confirmation of the theory in their experiments with artificial membranes; but it is difficult to verify its applicability to cell permeability because the formula contains several constants whose value for the cell is unknown. It must also be remembered that it is in no way essential for the Teorell-Meyer theory that the selectivity of the permeability of the cell be determined by the degree of dissociation of the colloids of the surface layer of the protoplasm (membrane), as the authors of the theory themselves thought. In this connection, one cannot but agree with the opinion of Frey-Wyssling (1950) that the protoplasm itself as a colloidal system has the conditions needed for Donnan equilibrium, so that the Teorell-Meyer theory is valid not only for the surface layers of the cell but also for the whole thickness of the protoplasm.

Thus, the Teorell-Meyer formula, often called the permeability formula, must be interpreted more widely: it refers not only to the conditions affecting the speed of movement of anions and cations in the surface layers of the cell, but also to the conditions of the movement of ions in the protoplasm as a whole.

This formula finds its most complete expression if account is taken of another factor, that is that the solubility of ions in the protoplasm can vary for different ions and can differ from their solubility in the solution surrounding the cell. This is achieved by introducing into the expression under the root in the numerator and denominator of the formula the product of the solubility of the ions ( $I_c I_a$ ) in the protoplasm. Then the "permeability" formula will become:

$$\frac{n_c}{n_a} = \frac{U_c[\sqrt{(4c^2 I_c I_a + A^2)} + A]}{U_a[\sqrt{(4c^2 I_c I_a + A^2)} - A]}.$$

The Teorell-Meyer theory has not found wide acceptance among physiologists because it does not take into account certain known facts which indicate that cells are simultaneously permeable for both anions and cations, their direction of motion being frequently towards the higher concentrations. It is true that Teorell (1937) tried to obviate this difficulty too. In his view, the factor responsible for the movement of the ions towards the higher concentrations is the diffusion potential. This arises as a result of metabolism and the production of electrolytes in the cell whose anions or

cations cannot pass through the membrane. This in its turn leads to a non-uniform distribution of the "passive electrolytes" present in the medium, to which the membrane is permeable. The greater the diffusion potential, the greater the inequality of the concentrations in the medium and the cell. Calculations show that the distribution of the "passive ions" must correspond to Donnan equilibrium: all the "passive ions" of like sign must be distributed

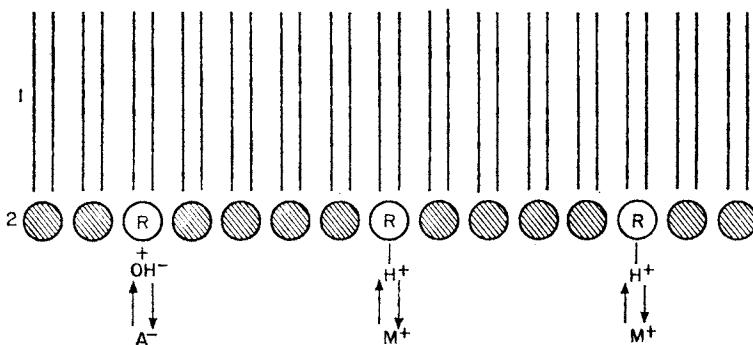


FIG. 5. Structure of the protoplasmic membrane according to Lundegårdh (1940).

1—micelles, 2—their radicals.

in like ratios and the accumulation in the cell of ions of like sign must lead to a corresponding reduction in the concentration of ions of the opposite sign. However, analysis of the contents of plant and animal cells radically contradicts this requirement of the theory (Fenn, 1936; Wilbrandt, 1938; Krogh, 1946, and others).

After the failure of Michaelis's theory and its several variants, Lundegårdh (1940) put forward another hypothesis for the membrane mechanism of the penetration of cations into the cell; this is now regarded as a step forward by comparison with the previous theories (Krogh, 1943, 1946). Lundegårdh thought that the surface of the cell is covered in palisade fashion by long molecules (or micelles) in a single or double layer. Some of these molecules have positive charges, but the greater part are negative, being distributed in a mosaic over the surface.

The mechanism of the penetration of cations into the cell is thought of as follows. First the ion attaches itself to the micelle, which is a component of the membrane; then the micelle turns through  $180^\circ$ ; thus the attached ion gets to the other side of the membrane—either inside or outside the cell, depending on which side the ion became attached (see Fig. 5). Lundegårdh's theory will be analysed in more detail below.

While he notes that Lundegårdh's hypothesis is in the highest degree speculative, Krogh (1943) none the less considers that it is the best of all the

current membrane theories, because it provides an explanation for a number of facts which the other membrane theories cannot explain. In applications to animal cells Krogh considers it possible to make the following additions to Lundegårdh's hypothesis.

He thinks that the micelles of which the membrane is composed have a chemical affinity either for sodium or potassium. He was compelled to resort to this supposition because in his own words: (1) any animal cell behaves like a typical osmometer; (2) water moves freely both into and out of the cell; (3) cells are permeable for anions and cations, both potassium and sodium being among the cations that penetrate the cell; (4) there is a sharp difference in the mineral composition of the cell and the medium. Krogh considers that, with these additions of his, Lundegårdh's hypothesis can explain all these phenomena.

Ussing (1947) and Levi and Ussing (1948) found by using radioactive sodium that in Ringer's solution the sodium ions in muscle fibres are quickly exchanged for the same ions in the surrounding solution; however, the sodium concentration in the muscle fibres does not change, remaining lower than in the medium. Starting from the Lundegårdh-Krogh hypothesis, they explain the exchange of sodium ions as follows. The micelles of the membrane, having a special chemical affinity to sodium, turn through 180° thanks to thermal movement and can thus come into contact now with the external, now with the internal medium of the cell. Thus, a sodium ion from the medium which has become attached to a micelle finds its way into the cell and replaces a sodium ion from within the cell; then the latter, after the next turn of the micelle, is on the other side of the membrane and so is exchanged for sodium from the medium.

This type of cell permeability is called by the authors "exchange permeability", because it is based on exchange adsorption and does not produce any change in the sodium concentration in the cell. The exchange of the cell's sodium ions for the sodium of the medium, according to this hypothesis, is similar to the phenomenon of self-diffusion — to the displacement of molecules in space in solutions in diffusional equilibrium. It must, however, be emphasised that the cell's ions are also exchanged for the same ions from the medium when their total concentration in the cell changes (on a change in the ion concentration in the medium, on the stimulation and damaging of cells, etc.).

To conclude this section of the chapter it must be said that all the cited membrane theories of cell permeability are of equal worth, because each of them can explain more or less satisfactorily one group of facts but cannot correctly explain the totality of facts relating to these phenomena. There are particularly many contradictions when the subject under discussion is the functional change in the cells, without accounting for which no theory of cell permeability can be built up.

At present, the membrane theory is being modified in the direction that

the penetration into the cell of a number of organic compounds and mineral substances and also their distribution between the cells and the medium is thought of as an "active process", controlled by the activity of enzymes localised in the protoplasmic membrane. The hypotheses relating to these modifications will be discussed in the course of our exposition of the experimental data on the permeability of cells for various substances.

A detailed exposition of the membrane theories of cell permeability has recently been made by Wartiovaara and Collander (1960), while Katchalsky (1961) has considered the permeability of membranes from the point of view of the thermodynamics of irreversible processes.

### *5. The Role of the Cell Envelope in Permeability Phenomena*

We have already mentioned that, in discussing the semi-permeable membrane, we should not be thinking of the cell envelope that, in certain cases, undoubtedly does exist and can clearly be seen under the microscope (the envelopes of plant cells, the sarcolemma of muscle fibres, cuticular rings, fertilisation sheathing, etc.). The proponents of the membrane theory deny the participation of these envelopes in cell permeability phenomena on the grounds that these formations cannot have the property of semi-permeability or selective ion permeability. In view of this, the presence on the surface of cells of these envelopes cannot be used to explain the osmometric behaviour of the cell, the sharp difference in mineral composition between the contents of the cell and the medium, the bioelectric properties of cells and other physiological phenomena. Besides, the existence of the envelopes is altogether dubious in a number of cases, and sometimes they are known not to exist (young plant and animal cells, many connective tissue cells, the protoplasts of plasmolysed cells and so on), whereas the presence of the membrane, from the point of view of the membrane theory, is essential for any cell. In Fig. 6 we show a scheme for the structure of the envelope of an erythrocyte, which shows clearly what the semi-permeable membrane should be taken to be.

Unfortunately, certain cytologists have confused cell envelopes with semi-permeable membranes. The founders of the membrane theory and most of their followers see the envelopes of plant and animal cells as protective formations, which have purely mechanical functions and let pass quite freely all solutes apart from suspensions of coarse particles (Overton, 1902a; Höber, 1926, 1945; Harvey and Danielli, 1939; Rumyantsev, 1939; Mitchison, 1952; Katz, 1956; Hodgkin, 1958, etc.).

In a number of cases the cell envelopes are morphologically well defined, having a different chemical composition and different physico-chemical properties from the inner layers of the protoplasm. They therefore provide different conditions for the diffusion of solutes from the remaining mass of the protoplasm. Many workers have observed that certain substances can ac-

cumulate in the cell envelopes in the first place and only then, depending on the functional state of the cell, penetrate the latter.

The role of the cell envelopes in permeability phenomena is, clearly, not limited to this. A good example of an envelope playing a protective role is the envelope of the eggs of horse ascarides and, probably, the eggs of other

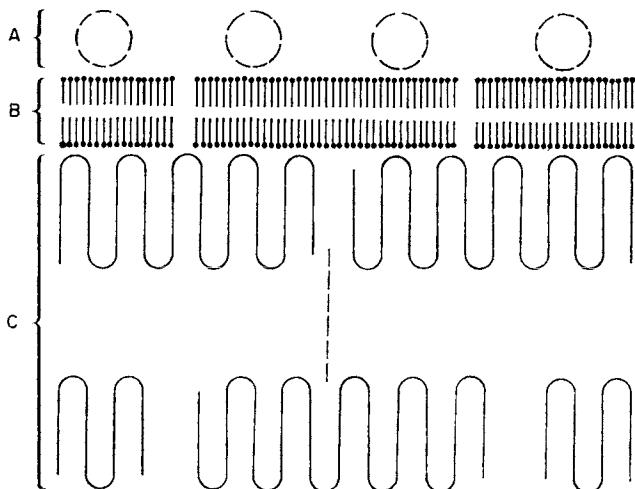


FIG. 6. The structure of the erythrocyte envelope (from the Russian translation of Mitchison, 1952).

A—layer of antigenic and isotropic proteins (20–50 Å); B—lipid membrane (40 Å); C—structural protein layer (5000 Å).

nematoda. Zavadovskii (1915) discovered that the eggs of horse ascarides develop both in pure water and in solutions of poisonous substances (copper sulphate, mercuric chloride), mineral acids and alkalis. The envelopes of the ascarides eggs are completely impermeable to copper sulphate and mercuric chloride and only with difficulty permeable to water. The embryos can live for a long time and move vigorously in poisonous liquids, so long as their covering envelope is not damaged. In solutions of organic substances (alcohol, acetic acid), which precipitate proteins and dissolve lipoids, the envelope is broken down and the solutes penetrate the eggs and kill the embryo.

#### *6. Conclusions*

1. The classical membrane theory of cell permeability starts from the idea that any animal and plant cell is an osmometer. From this point of view, the contents of the cell are thought of as a simple aqueous solution of colloids and crystalloids which develop an osmotic pressure equal to that of the medium.

2. Under these circumstances, there must be on the surface of the cell a special layer (the protoplasmic membrane, plasmolemma or semi-permeable membrane) separating one aqueous solution (the contents of the cell) from another aqueous solution (the surrounding medium). The protoplasmic membrane must pass molecules of the solvent (water) without any difficulty and pass selectively the molecules of solutes.

3. The proponents of the classical membrane theory, starting from the idea of the cell as an osmometer, were compelled to assert that such important substances for life as sugars, amino acids, strong electrolytes, etc., should either not penetrate the cell at all or only exceedingly slowly. This assertion contradicts the biological sense of permeability phenomena.

4. All the variants of the membrane theory (Overton's lipoid theory, Nathansohn's mosaic theory, the Ruhland-Michaelis ultrafilter theory and the Collander-Höber lipoid filter theory) have not been experimentally verified and have been abandoned by many physiologists as being contradictory to the facts.

5. At present the classical membrane theory is being modified in the direction that the cell membrane is thought of not as a passive, but as an active formation. In the majority of cases cell permeability is treated as the "active transport of substances", controlled by enzymic mechanisms in the membrane.

## CHAPTER II

# Do Live Cells Have Osmometric Properties?

### *1. Plant Cells*

The experiments of Pfeffer (1877) and deVries (1884) with plant cells, as has already been mentioned above, laid the foundation of the membrane theory. The writings of these authors gave a definite impetus to the renowned theory of solutions of Van't Hoff (1887) and Arrhenius (1887) and at once won wide recognition.

Pfeffer and de Vries discovered that plant cells with a central vacuole behave like osmometers in solutions of a number of substances: their volume ( $V$ ) changes in inverse proportion to the osmotic pressure of the medium ( $P$ ). They found that the following relation holds between the volume of the cell and the concentration of the substance in the solution ( $C$ ):

$$VC = V_1 C_1 = \text{const.}$$

Subsequently, the applicability of this formula to vacuolar plant cells was demonstrated by numerous workers.

The experiments described do not yet prove, however, whether the part of the semi-permeable cell membrane or osmometer is played by the tonoplast or the protoplasm as a whole. Pfeffer and de Vries supposed that the membrane was localised on the surface of the cytoplasm. The testing of this hypothesis was carried out by Höfler.

Höfler (1918) developed the plasmometric method of studying the permeability of plant cells: this method is based on the separate measurement of the volumes of the vacuole and the cytoplasm. The method was subsequently used by Höfler (1930, 1931, 1932, 1934, 1939) and Hüber and Höfler (1930) to discover that in solutions of various substances the change in volume of the vacuole obeys the law of osmosis, while the change in volume of the cytoplasm does not. On the basis of convincing evidence Höfler came to the conclusion that many so-called osmotically active substances which cause plasmolysis freely penetrate the outermost layer of the cytoplasm, but either do not penetrate the tonoplast or penetrate it only with difficulty.

In view of this, the term "permeability" (Permeabilität) was taken by Höfler (1931) to signify only the passage of a substance through the cytoplasm into the vacuole, while he proposed that the term "intrusion" (Intrabilität) should be used to denote its passage into the cytoplasm.

Further, Chambers and Höfler (1931) showed on isolated vacuoles of plant cells that such vacuoles with clearly visible tonoplast did indeed behave like osmometers in hypertonic solutions of a number of substances: the vacuolar volume changed in inverse proportion to the change in the osmotic pressure of the medium. These facts allowed them to say that the water in the vacuoles of plant cells is retained, in all probability, by osmotic forces, but that the osmotic mechanism for regulating the water balance is not applicable to the cell as a whole, because change of the water content in the cytoplasm does not obey the law of osmosis.

Additional support for this is to be found in the fact that, in hypertonic solutions of a number of substances, principally electrolytes, there sometimes occurs a sharp reduction in the volume of the vacuole together with a swelling of the cytoplasm, which it is quite impossible in some cases to separate from the cell envelope (Plowe, 1931 a, b; Strugger, 1932; Ullrich, 1939, etc.).

In the excellent work recently published by Wartenberg (1957, a, b; 1958) it has been shown that in hypertonic solutions of potassium chloride and certain sugars the central vacuole is reduced in volume, but that, as a rule, the outermost layer of the protoplasm is not separated from the cell envelope and new vacuoles are formed in large numbers in the cytoplasm.

The enormous amount of material on the so-called "osmotic properties" of plant cells has recently been analysed by Maksimov (1946). As a result of this analysis the author came to the conclusion that the classic (i.e., osmotic) theory of the regulation of the entrance of water into plant cells cannot be substantiated. Referring to the literary sources, he produces the following interesting data (Table 4).

TABLE 4. THE OSMOTIC PRESSURE (IN ATMOSPHERES) IN THE LEAF CELLS  
OF VARIOUS PLANTS  
(after Maksimov, 1946)

	Beech	Garden beet	Wild grape	Cotton plant
Whole cell (plasmolytic data)	30-32	14	15-16	22
Protoplasmic sap	18-24	—	25	17-18
Vacuolar sap	5-8	7	5-7	9-10

It follows from the table that the osmotic pressure of the vacuolar fluid is two to five times less than the pressure of the protoplasmic fluid. From the standpoint of the osmotic theory, it is impossible to understand how the water moves from the protoplasm to the vacuole or why it does not come out of the vacuole into the protoplasm. These experiments can be used as yet one more proof that a plant cell as a whole does not behave like an os-

motic cell. Further, Maksimov and Mozhayeva (1944 a, b), Mozhayeva (1947, 1950 a, b), Alekseyev (1948), Rogalev (1949), Shcherbakov and Semiotrocheva (1953), Alekseyev and Gusev (1957) and others have shown that the absorption of water by plant cells and their water-holding power depend on the hydrophilic properties of the cell colloids, which, in their turn, are closely connected with metabolism.

Consequently, osmotic experiments with plant cells which have a central vacuole cannot reveal the presence of an osmotically active membrane on the surface of the cytoplasm and cannot be used as proof that the quantity of water in the protoplasm is regulated by osmotic forces, a fact which is admitted by the supporters of the membrane theory themselves. Rubinshtein (1939) had the following to say on this topic: "... we are compelled to disregard completely in our calculations all results obtained by the osmotic method in the study of plant cells, results which historically, as is well known, were the basis of all contemporary teaching on the semi-permeable plasma envelope" (p. 9).

To decide the question of the existence of a semi-permeable membrane Rubinshtein supposes that osmotic experiments with animal cells may be used, as these have no vacuole. The problem can, however, be solved equally well in non-vacuolar plant cells. Experiments of this kind were done by Walter (1923). He placed non-vacuolar cells of sporogenic fibres of the alga *Lemanea* in solutions with various concentrations of sugar and found that the change in volume of the cells did not obey the law of osmosis. Their volume in these solutions changes in the same way as the volume of gelatine, casein, nucleine, starch, etc., changes in sugar solutions. On the basis of his experiments Walter came to the conclusion that the absorption and emission of water by non-vacuolar cells is not an osmotic, but a colloidal, phenomenon, connected with the change in the hydration of the protoplasmic colloids.

## 2. Animal Cells

Animal cells also do not behave like osmometers.

Overton (1902a) lists some interesting observations by Hales (1732) and Nasse (1869), which, as he writes, struck him with the idea of the similarity of the osmotic properties of animal and plant cells. Hales observed that, on the introduction into the blood of warm-blooded animals of a large quantity of water, the muscles, liver, kidneys and other organs swell violently. This phenomenon rapidly disappears on the introduction into the blood of a solution of sodium chloride in the concentration 1: 80. Nasse's experiments indicate that isolated frog muscles remain alive for a long time in a 0·6 per cent saline solution without changing their external appearance. Nasse noticed that the muscles remained excitable for a long time in all concentrations of this salt from 0·3 to 1·25 per cent, the most favourable being that in which the muscles neither swelled nor gave up water.

Overton determined by weighing the loss or absorption of water by isolated frog muscles in solutions of a wide variety of substances and came to the conclusion that muscle fibres (except for their sarcolemma) must be considered as osmotically active formations. It was in this sense also that he interpreted the data of Hales and Nasse.

Meanwhile, there was no correspondence in Overton's experiments between the osmotic law and the change in weight of the muscles: in hypertonic solutions the muscles lost water, and in hypotonic solutions absorbed less than was to be expected if the muscle fibres obeyed this law. To eliminate this contradiction, Overton, as is well known, assumed that a considerable amount of the water in the muscles (about 35 per cent) is in the bound state and takes no part in the osmotic processes.

However, Fenn (1937a), using the same method, found that there is very much less bound water in frog muscles.

Hill (1930) studied the change in volume of frog muscles in various media and found double the divergence from the osmotic law. He tried to explain this divergence not by the presence of bound water in the muscles, but by the fact that in the preparation of the muscles part of their fibres lost their osmotic properties.

Further, Hill and Kupalov (1930) calculated the amount of all the substances they considered to be osmotically active (mineral ions, amino acids, creatine phosphate, etc.) present in the muscles and blood plasma of a frog. The concentrations of the "osmotically active" substances in the blood plasma and the muscles turned out to be the same and equal to their concentration in a physiological solution. Further, Hill (1930), using a specially constructed thermo-couple, compared the vapour tension of a physiological solution and solutions of the various substances in which the muscles were immersed (until diffusional equilibrium was established). On the basis of the data obtained he concluded that 95 per cent of all the water in the muscles is free and constitutes a "free solvent". From these experiments it follows that a muscle fibre should behave like a perfect osmometer, because almost all the water in it is a normal solvent, while all the principal mineral substances in the muscle (primarily the potassium and phosphate ions) should be soluble in this water. Only in this case can the osmotic pressure inside the fibre be equal to the osmotic pressure of the medium. Hill (1930, 1935) supposes that the same occurs in erythrocytes.

The principle of the equality of the osmotic pressure within the cell and in the medium surrounding it, which is postulated by the membrane theory, finds confirmation also in later work of many students of this problem (Fenn, 1936; Boyle and Conway, 1941; Krogh, 1946; Conway and Hingerty, 1946; Conway, 1957; Hodgkin, 1958; Dick, 1959 etc.). It is established by chemical methods that the total molar concentration of all the known substances within the cell is approximately equal to their molar concentration in the external fluid.

According, however, to the data of other workers (Opie, 1949; Robinson, 1950, etc.), the overall concentration of osmotically active particles in the cell is considerably higher than in the external medium. In this connection a number of authors suppose that there is in cells a special mechanism which continuously pumps out the water entering the cell and protects it from swelling. This suggestion is based on the fact that sections of animal organs in an isotonic salt medium swell strongly in anaerobic or low temperature conditions or in the presence of cyanides, but when these same sections are returned to normal conditions they again lose water (Stern *et al.*, 1949; Opie, 1949, 1954; Aebi, 1951, 1953; Robinson, 1952, 1953, 1954; Mudge, 1951a, b, 1953; Whittam and Davies, 1953; Bartley *et al.*, 1954; Cort and Kleinzeller, 1956).

In a series of articles Conway and his colleagues (Conway and McCormack, 1953; Conway and Geoghegan, 1955; Conway, Geoghegan and McCormack, 1955) published the results of some chemical and cryoscopic studies which contradict the ideas of Opie, Robinson and others. These data show that the molar concentration and freezing point of organs previously frozen in liquid oxygen and crushed are the same as those for blood plasma, if these organs, when ground in the frozen state, are kept for some little time at 0°. If the fluid is kept with the powder from these organs at 37°, an increase in the molar concentration is to be observed accompanied by a lowering of the freezing point, which is explained by the hydrolysis of a number of the labile organic compounds in the cells. Leaf and his colleagues (Leaf, 1956; Leaf *et al.*, 1954) come to a similar conclusion. We shall try to show below that all the contradictory data quoted above can best be explained from the point of view of a colloido-chemical mechanism for the regulation of the water content of cells rather than from osmotic laws.

Erythrocytes are often cited as an example to prove that the regulation of cell water is achieved by osmotic forces: these cells appear to react very nicely to any change in the osmotic pressure of the external medium (Rubinshstein, 1939). Jacobs (1932), Ponder (1934, 1936, 1940), Ørskov (1935), Widdas (1953, 1954) and many other workers assert that the erythrocyte is the perfect osmometer, consisting of a membrane and containing haemoglobin, salts and other substances in an aqueous solution. Correspondingly, advocates of the membrane theory see the cause of haemolysis in the destruction of the erythrocyte membrane. The change in volume of erythrocytes or their degree of haemolysis is often used to draw conclusions about the speed of penetration into blood cells of water and water-soluble substances (Jacobs and Parpart, 1931; Jacobs, 1932, 1934, 1938, 1954; Höber and Ørskov, 1933; Parpart, 1936; Ørskov, 1935; Parpart and Jacobs, 1936; Jacobs, Glassman and Parpart, 1936, 1950; Meldahl and Ørskov, 1940; Jacobs and Willis, 1947; Willis, Love and Jacobs, 1947; Jacobs, Stewart *et al.*, 1949; Love, 1953; Widdas, 1953, 1954, etc.).

To make the change in volume of erythrocytes correspond to the law of

osmosis, it is assumed that about half of all the water in them is in the "bound" state (Ponder, 1936, 1940). But even if such an assumption is made, the desired results are not obtained, because the correction for the so-called "osmotically inert volume" (the "bound water" plus the dry residue of the cell) should be made individually for each of the substance dissolved in the medium that "does not penetrate" the cell and for every concentration of these substances.

Thus, in his book Ponder (1934, p. 119) produces data on the increase in volume of the erythrocytes of a rabbit in relation to the dilution of solutions of sodium chloride, potassium chloride and glucose, in which these cells were placed. To make these data correspond with the requirements of the osmotic theory, Ponder calculated how much free (osmotically active) water there should be in the erythrocytes and found that this quantity is not constant but varies depending on the concentration of the substance in the solutions (sodium chloride from 30 to 50 per cent, potassium chloride from 27 to 66 per cent, glucose from 20 to 36 per cent).

Further, Ege (1921a), showed by osmotic experiments that rabbit erythrocytes are apparently completely impermeable for glucose, sodium chloride and potassium chloride. But when he compared the volumes of the cells in isotonic solutions of these substances, he found that they differ, a fact which cannot be reconciled with the law of osmosis. Another writer, Krevisky (1930), used haematoctests to measure the diminution of human, sheep and rabbit erythrocytes in solutions of sodium chloride. He also failed to find any correspondence between the change in volume of these cells and the law of osmosis.

Analogous experiments were performed by Schiodt (1931) with dog erythrocytes and Ørskov (1946a) with human erythrocytes and showed similar results. They showed that the correction for the osmotically inert volume in the cell (the correction  $b$  in the equation  $p(v - b) = \text{const.}$ ) changes depending on the osmotic pressure of the medium. Schiodt's data are given in Table 5.

TABLE 5. THE CHANGE IN VOLUME OF DOG ERYTHROCYTES IN RELATION

TO THE OSMOTIC PRESSURE OF THE MEDIUM

(from Schiodt, 1931)

Concentration of NaCl (in %)	Osmotic pressure ( $p$ )	Volume of erythrocytes ( $v$ )	Size of the osmotically inert volume ( $b$ )
0·91	0·56	100·0	—
1·67	1·00	75·0	43·0
5·00	2·95	62·4	53·6
6·70	4·95	68·2	64·0
10·00	5·90	67·4	64·0

The author of the article is uncertain why 57 per cent of the volume of the cells is osmotically active (i.e., almost all the water of the cell) at low concentrations of sodium chloride, while at high concentrations only 36 per cent is active, i.e., almost as little as half of the cell water.

In a study of osmotic equilibrium in human erythrocytes in which an immersion refractometer was used, Dick and Lowenstein (1958) obtained data that suggested that erythrocytes do not behave like perfect osmometers.

To study the permeability of cells for water and water-soluble substances many osmotic experiments were conducted on the eggs of marine animals (McCutcheon and Lucké, 1927, 1932; Lucké and McCutcheon, 1927; Lucké, 1931, 1940; Stewart, 1931, a, b, 1932; Stewart and Jacobs, 1932, 1936; Lucké, Larrabee and Hartline, 1935, 1936; Lucké, Ricca and Hartline, 1936; Lucké, Hartline and Ricca, 1939).

Experiments with the eggs of the sea urchin *Arbacia punctulata* show that the volume of these eggs in sea water diluted to various degrees with distilled water changes in apparently complete agreement with the osmotic law:  $p(v - b) = \text{const}$ . In this case the correction  $b$  is taken to be 11 per cent of the volume of the cells (see Table 6).

TABLE 6. THE CHANGE IN VOLUME OF THE EGGS OF *Arbacia punctulata* IN SEA WATER DILUTED WITH DISTILLED WATER. THE INITIAL PRESSURE OF THE SEA WATER ( $p$ ) IS TAKEN AS UNITY  
(from Lucké, 1940)

Relative pressure of the medium ( $p$ )	Observed volume of the egg ( $v$ )	$pv$	$p(v - b)$
1·0	2·656	2·656	2·368
0·9	2·898	2·608	2·364
0·8	3·170	2·536	2·319
0·7	3·679	2·575	2·386
0·6	4·232	2·539	2·377

If we take into account the fact that the egg cells of this animal contain 18·1 per cent dry residue (Page, 1927), we find that all the water of the egg cells is free. Hence follows the improbable conclusion that the protoplasmic colloids in this case behave like hydrophobic substances. Again, Lucké (1940) finds a much greater value for the correction  $b$  for the eggs of other animals. Thus, for the eggs of the annelid *Chaetopterus pergamentaceus*, the molluscs *Cumingia tellenoides* and *Ostrea virginica* the correction  $b$  ranges from 30 to 50 per cent of the volume of the eggs. In the author's opinion, the protoplasm in this case contains a considerable quantity of bound water.

According to the measurements of Shapiro (1948), the correction  $b$  for unfertilised eggs of *Arbacia punctulata* is 7·3 per cent rising immediately after fertilisation to 27·4 per cent, while the change in volume of the eggs in sea water on fertilisation does not exceed 4 per cent.

The egg cells of various marine invertebrates have also been the subject of osmotic experiments by other workers (Ephrussi and Neukomm, 1927; Bialaszewicz, 1929, 1933; Dorfman, 1933, etc): but these authors did not succeed in finding complete agreement between the change in volume of the cells and the law of osmosis.

According to the data of Ephrussi and Neukomm for the eggs of *Paracentrotus lividus* the correction b should be equal to 46 per cent of the volume of the cells.

Since the eggs of this animal contain 77.3 per cent water, we see that they should contain about 30 per cent bound water, which does not take part in the osmotic processes, while in the eggs of *Arbacia punctulata*, which contain about the same amount of water (81.9 per cent), according to the data of Lucké, there is no bound water.

Finally, after the thorough work of Aizenberg (1939), in which he studied the change in volume of the unfertilised eggs of echinoderms and sea worms in solutions of various non-electrolytes, it may confidently be stated that one cannot adopt a correction for the bound water of the magnitude needed to make the experimental data agree with the osmotic law.

In this connection the experiments of Vasil'yev (1922) are of great interest. He used an ocular micrometer to measure the thickness of a nerve in solutions of various mineral salts, acids and bases. He established that in hypertonic solutions of various substances (isotonic one to another) the volume of the nerve changes in different ways. In salt solutions the nerve is compressed, while in acidic or basic solutions it expands. This expansion of the nerve is not as great as in distilled water. The addition of a neutral salt to an acidic solution into which the nerve has been placed inhibits the swelling of the nerve. In order of effectiveness the salts can be placed in the following series: potassium chloride is less effective than sodium chloride, and the latter less than calcium chloride. Bivalent cations suppress the effect caused by univalent cations. On the basis of these experiments, Vasil'yev came to the conclusion that "the facts enumerated cannot possibly be explained from the standpoint of the osmotic theory, while, on the other hand, they are in complete and detailed agreement with the features of colloid chemistry" (p. 285).

The problem of water equilibrium in cells has been studied in detail by Nasonov and his colleagues. Thus, Nasonov and Aizenberg (1937) investigated the change in volume of frog leg muscles in solutions of a number of non-electrolytes, prepared in Ringer's solution: urea, glycerol, alanine, glucose, galactose, sucrose, lactose, dextrin, gum arabic, egg albumin and peptone. Of these substances only urea and glycerol, Overton (1902a) asserted, penetrate the muscle fibres, while dextrin, gum arabic, egg albumin and peptone appeared not to pass through even the envelope of connective tissue that clothes the muscle as a whole.

The authors also put frog muscles in solutions of various concentra-

tions and studied the process of dehydration during the period up to the establishment of water equilibrium (see Figs. 7 and 8). This process ends in 2 to  $2\frac{1}{2}$  hr. They also studied the relation of the change in volume of the muscles and the concentration of the non-electrolytes in the surrounding solution under water equilibrium conditions (Figs. 9 and 10).

According to the membrane theory, the curves of muscle dehydration against time (Figs. 7 and 8) show that all the non-electrolytes tested by the authors appear not to penetrate the muscle, because the muscles in solutions of these substances maintain their water loss. In this case, the muscles should lose an identical amount of water in isomolar solutions, which does not in fact happen. It was found that in isomolar solutions under equilibrium

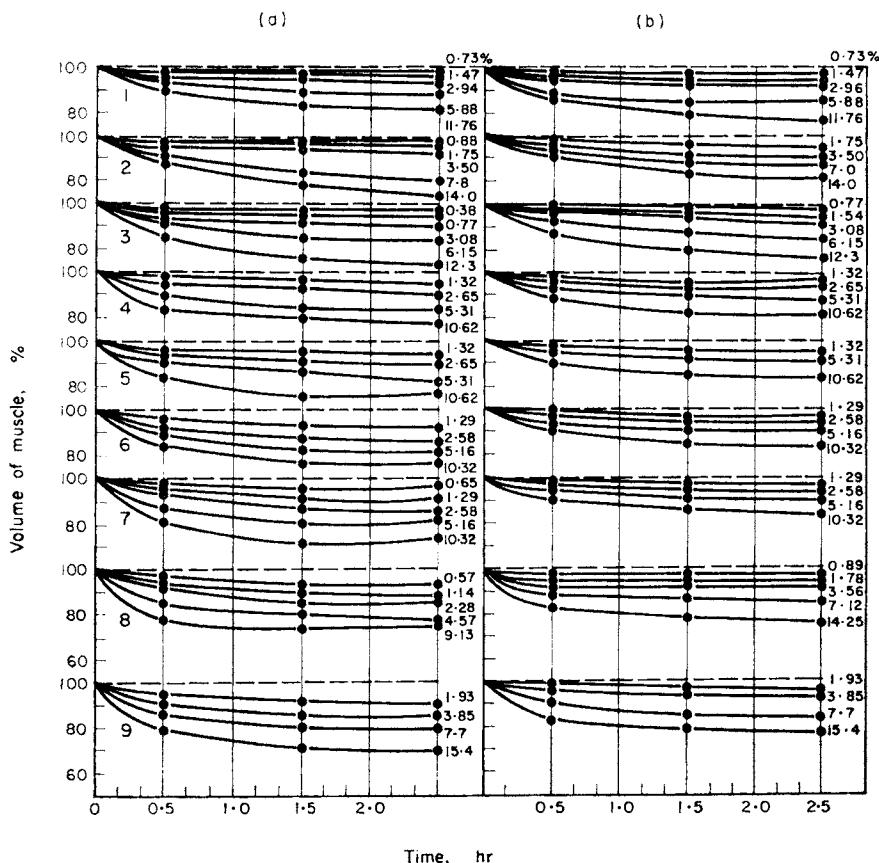


FIG. 7. The change with time in the relative volume of live (a) and dead (b) muscle in the presence of non-electrolytes (concentrations are volumetric and are shown on the right of each figure).

(From Nasonov and Aizenberg, 1937).

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

conditions the muscles lose different amounts of water. In order of increasing effectiveness in removing water, the substances can be placed in the following series: urea, glycerol, alanine, glucose, galactose, sucrose, lactose, dextrin, gum arabic, egg albumin. It can be seen from this series that the ability of substances to reduce the muscular volume increases with increasing

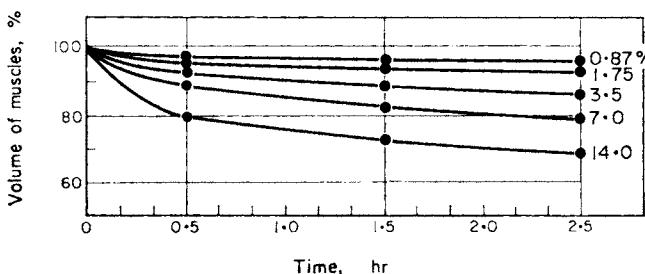


FIG. 8. The change with time in the relative volume of live muscle in peptone solutions (concentrations are volumetric). (From Nasonov and Aizenberg, 1937).

molecular weight. This is in radical disagreement with the requirements of the membrane theory. Further, in solutions of urea and glycerol, which are known to permeate cells with ease, the muscles in the equilibrium state should not suffer the least reduction in volume, whereas in a 1 M urea solution they lose 18 per cent of their water. In solutions of alanine and sugars, which are supposed not to penetrate the muscles, the muscles should suffer equal reductions in volume: but this does not happen. In this way Nasonov and Aizenberg established that in sugar solutions the muscles lose water 2.5 times less than the osmotic theory requires.

In their experiments with substances of large molecular size (dextrin, gum arabic and albumin) Nasonov and Aizenberg found that the muscles were deprived of water to a very much greater extent than the osmotic theory requires. This phenomenon can in no wise be explained by the presence of bound water in the cells, because the correction for the "osmotically inert volume" is introduced in just those cases where the cells lose less water than they should according to the osmotic law.

The authors also established that the reduction in volume of the muscles is determined not by the molar concentration of the non-electrolytes, but by the volume they take up in the solution: it was found that concentrations of equal volume caused equal reductions in the muscular volume. The same is found in experiments with dead muscles (see Figs. 9 and 10). The same law was later found by Nasonov (1938) for the case of gelatine and live muscle and by Aizenberg (1939) in experiments with the eggs of sea animals (*Pectinaria hyperborea* and *Asteria rubens*).

It can be seen from the curves in Figs. 11, 12 and 13, which have been taken from the above mentioned articles by Aizenberg, that the non-electrolytes

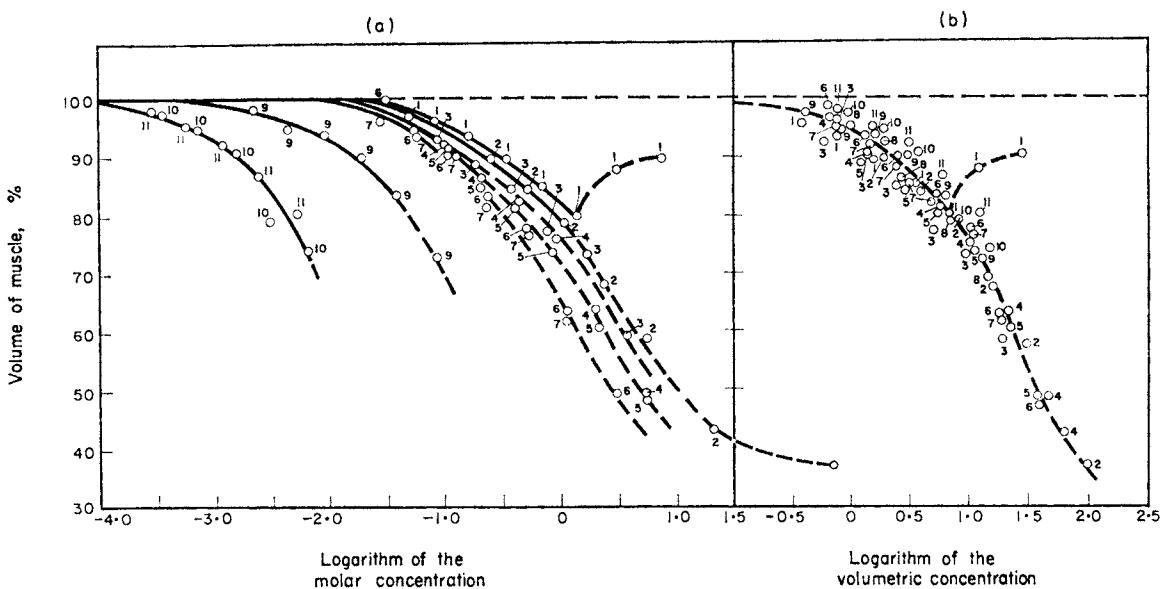


FIG. 9. The effect of non-electrolytes on the volume of live muscle.

(From Nasonov and Aizenberg, 1937).

1—urea; 2—glycerol; 3—alanine; 4—glucose; 5—galactose; 6—sucrose; 7—lactose; 8—peptone;  
9—dextrin; 10—egg albumin; 11—gum arabic.

In Fig. a, the unbroken lines denote the range of concentrations in which the muscle remained excitable.

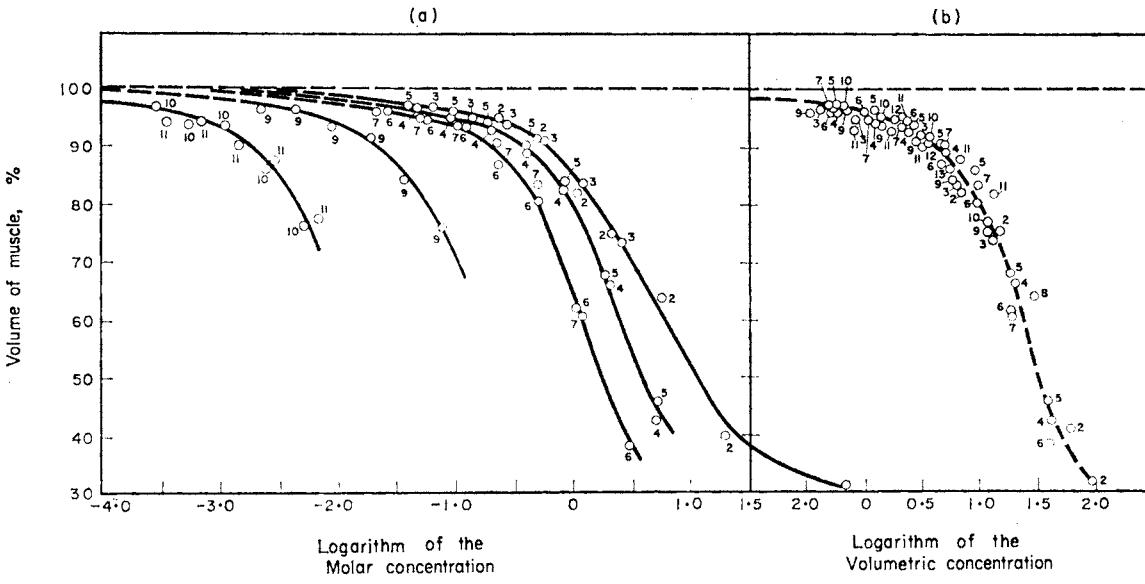


FIG. 10. The effect of non-electrolytes on the volume of dead muscle (from Nasonov and Aizenberg, 1937).

2—glycerol; 3—alanine; 4—glucose; 5—galactose; 6—sucrose; 7—lactose; 9—dextrin; 10—egg albumin;  
11—gum arabic.

tested by her can be placed in the same order of effectiveness in removing water as was obtained by Nasonov and Aizenberg in their muscle experiments, that is (in order of decreasing effectiveness): dextrin, lactose, sucrose, glucose, galactose and glycerol. In concentrations of equal solute volume (Figs. 12b and 13b), egg cells, just like muscles, lose equal amounts of water in solutions

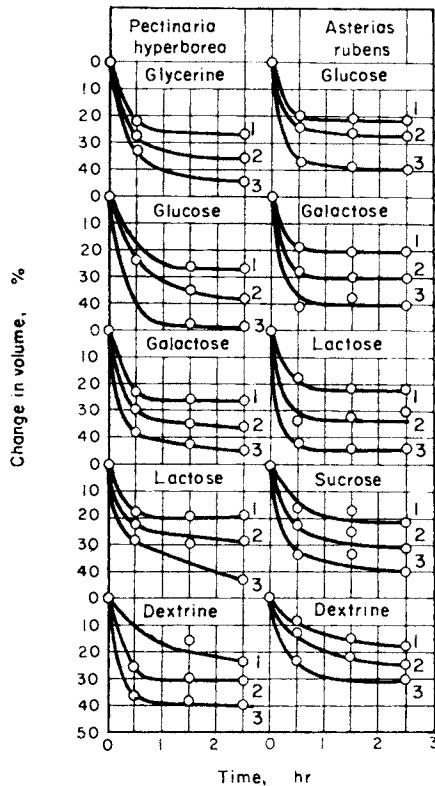


FIG. 11. The change with time in the volume of egg cells in relation to the concentration of non-electrolytes in the medium (from Aizenberg, 1939).

Concentration of the non-electrolytes: 1—2.5 per cent; 2—5.0 per cent;  
3—10 per cent.

of all the substances tested; this is in absolute disagreement with the osmotic law, because solutions of equal solute volume are not equal in molarity, but it is only in isomolar solutions that egg cells could lose equal amounts of water if the water was held in them by osmotic forces, as happens in the case of an osmometer. It should be noted that the cells remained indubitably alive throughout the whole duration of Aizenberg's experiments, because on subsequent transfer to sea water they were fertilised and began to divide.

On the basis of the data obtained Nasonov and Aizenberg (1937) came to the conclusion that "... the removal of equal amounts of water in concentrations of equal solute volume is possible only when the solute penetrates into the muscle" (p. 183).

The correctness of this proposition can be proved experimentally by comparing the relation in time between the dehydration of cells and the absorption by them of the substances causing their dehydration. To this end I performed several series of time experiments comparing the penetration of a substance into the cells with the change of the quantity of water in them (Troshin, 1953).

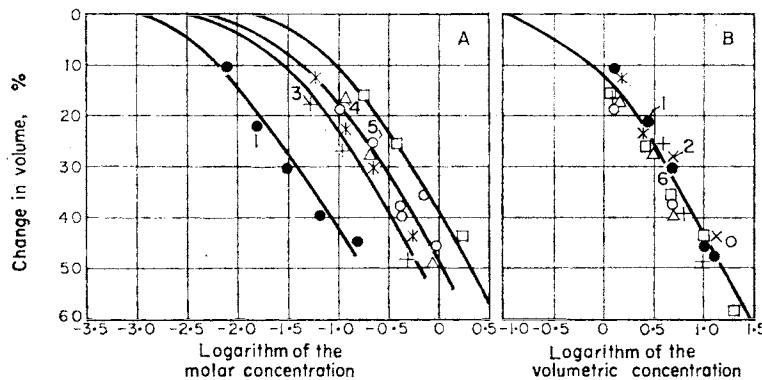


FIG. 12. The change in volume of egg cells of *Pectinaria hyperborea* in relation to the concentration of non-electrolytes in the medium (from Aizenberg, 1939).

1—dextrin; 2—lactose; 3—sucrose; 4—glucose; 5—galactose; 6—glycerol.

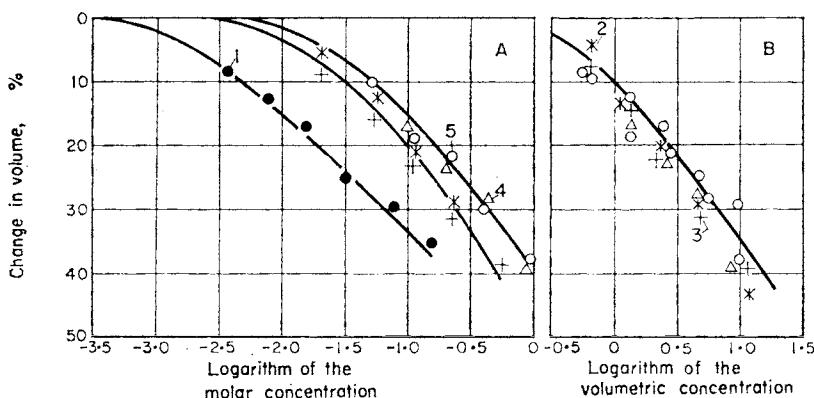


FIG. 13. The change in volume of egg cells of *Asterias rubens* in relation to the concentration of non-electrolytes in the medium (from Aizenberg, 1939).

1—dextrin; 2—lactose; 3—sucrose; 4—glucose; 5—galactose.

In the first group of experiments the change in volume of rabbit erythrocytes in galactose solutions was determined in relation to the penetration of this sugar into the cells.

All the galactose solutions were prepared in Ringer's solution. Fig. 14 shows the change in volume of the erythrocytes in a 2 per cent solution of galactose and the penetration of the galactose into the cells with time. It can be seen from the figure that, as more galactose penetrated the erythrocytes, there was

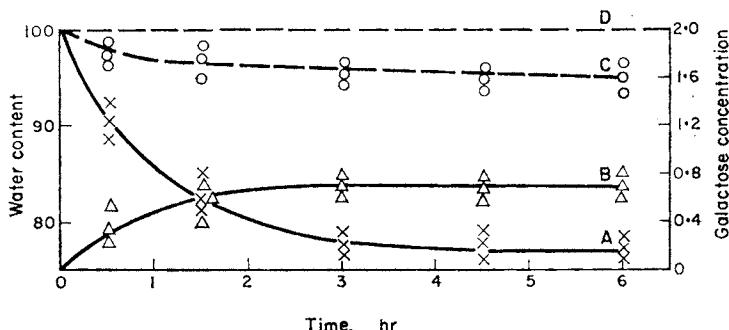


FIG. 14. The change with time in the water content (expressed as per cent of control) and galactose content (in per cent) in rabbit erythrocytes placed in a 2 per cent solution of galactose made up in Ringer's solution.

A—decrease of the erythrocyte water content; B—absorption of galactose by the erythrocytes; C—decrease in the galactose in the medium; D—initial water content of the cells.

a simultaneous reduction in the amount of water in them. In 3 hr complete diffusion equilibrium was established between the galactose that had penetrated the erythrocytes and the galactose in the external solution. In the same period complete water equilibrium is also reached between the cell and the medium.

These results, in my view, remove the last traces of doubt about the erroneous nature of the idea of erythrocytes as "perfect osmometers" that has been entertained by many writers. In fact, if the distribution of the water in the present case were regulated by osmotic forces, the curve of the loss of water by the cells with time would have to be regarded as proof of the impermeability of erythrocytes for galactose, which is not the case. If, on the other hand, this substance which causes the cells to lose water penetrates them, then, from the point of view of the osmotic theory, the water content of the erythrocytes must inevitably revert to its original level, which is also not the case. In fact, precisely the opposite phenomenon is found: as more sugar penetrates the cell, there is a simultaneous loss of water by the erythrocytes. Consequently, the erythrocytes lose water not because the sugar does not penetrate them, but because it does.

In the next series of experiments the subject studied was the relation between the water content of the cells and the galactose concentration in the surrounding solution in erythrocytes in conditions of complete diffusion and water equilibrium. As can be seen from Fig. 14, this equilibrium state is attained in  $3\frac{1}{2}$  hr. The time of all the experiments to be mentioned below for all concentrations was 4 hr, that is, equilibrium can be taken to have been established for certain.

The data found in this series of experiments are set out in Fig. 15. Here the curve A reflects the relation between the water content in the erythrocytes and the concentration of galactose in the medium that would hold if the erythrocytes behaved like osmometers, while curve B gives the experimentally determined relation.

Curve B shows that the erythrocytes lose water as the concentration of galactose in the external medium increases. In the early part of the curve (up to 1 per cent solution) this curve coincides exactly with the theoretical curve calculated from the law of osmosis; but further on this coincidence is no longer observed: the divergence between the curves A and B increases continuously with increasing sugar concentration in the medium. If only

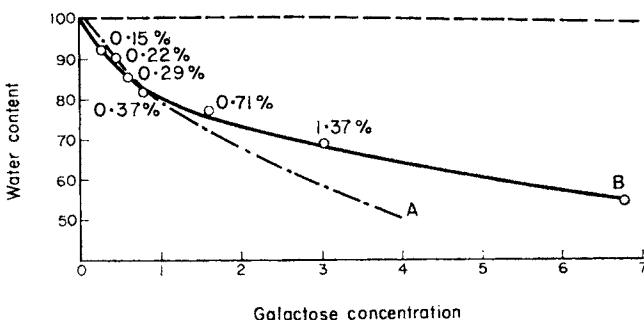


FIG. 15. The relation between the water content of erythrocytes (expressed as per cent of control) and the galactose concentration (in per cent) in the surrounding medium.

A—theoretical curve, calculated on the basis of the osmotic law; B—curve of the dehydration of the erythrocytes, obtained experimentally. The figures over the points on curve B denote the sugar concentration in the erythrocytes (in g/100 ml cell water).

the first part of the curves is taken into consideration, it is possible to form the impression that erythrocytes behave like perfect osmometers. But this impression will be erroneous, because the galactose is simultaneously penetrating the erythrocytes. The greater the concentration of sugar in the medium, the greater is the loss of water from the erythrocytes and the concentration of galactose into them. Thus, when the concentration of galactose in the medium is 3.07 per cent, its equilibrium concentration in the erythrocytes is

1.37 per cent (this calculation was made in grams per 100 g of water in the erythrocytes). Under these conditions, the erythrocytes lost 31 per cent of their water. When the galactose concentration in the medium was 0.62 per cent and in the cells 0.29 per cent, the amount of water in the erythrocytes was reduced by 15 per cent. These data show that the coincidence between the experimentally observed dehydration curve and that calculated on the basis of the osmotic law is accidental, that there is no causal relation between

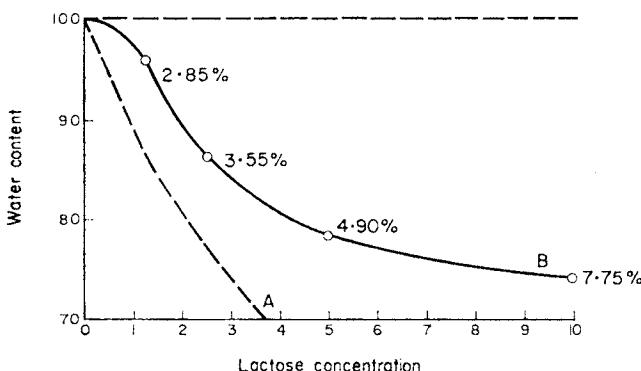


FIG. 16. The relation between the water content of yeast (expressed as per cent of control) and the lactose concentration (in per cent) in the surrounding medium.

them. All the coincidence indicates is that the order of magnitude of the dehydration of the cells is about the same as if osmotic laws lay at the foundation of the phenomenon.

The next series of experiments was done to determine the relation between the degree of dehydration of yeast cells and the concentration of lactose in the medium and in the cells in conditions of diffusion equilibrium.

The yeast cells used in the experiments were *Saccharomyces ellipsoideus*, grown in beer wort and washed three times in Ringer's solution. The yeast cells were placed for 3 hr in the lactose solutions, which were made up with Ringer's solution, after which the quantity of water and lactose in them was determined. The results are shown in Fig. 16.

Here we see the same law as was found in the erythrocyte experiments, namely: the more lactose passes into the yeast cells, the more water they lose. Thus, when the lactose concentration in the medium is 1.25 per cent, the cells lose 4 per cent of their water. This occurs at a time when the cells contain 2.85 per cent of this sugar. When the lactose concentration in the medium is 10 per cent, there is 7.75 per cent in the cells, which, in the case of yeast cells, lose 25.6 per cent of their water under these conditions. The analysis at other concentrations gives similar results.

We should also reach similar conclusions about the mechanism for regulating the water content in muscle fibres. It is easy to satisfy oneself that

this is so by juxtaposing the published data available relating to the change in volume of muscles in solutions of various substances and the distribution of the latter between the muscle fibres and the medium.

As an example let us take the results of the experiments reported in the above mentioned article by Nasonov and Aizenberg (1937). The authors determined the dehydration with time of muscles placed in solutions of various substances and their dehydration in relation to the concentration

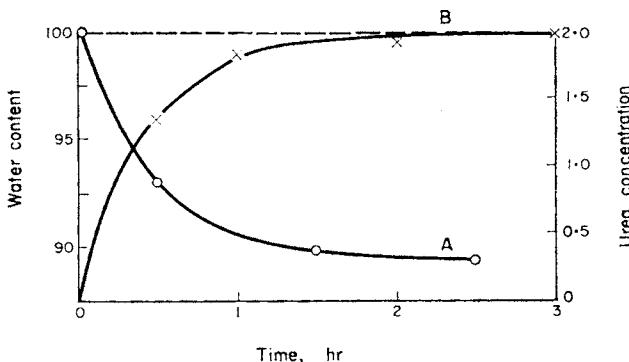


FIG. 17. The change with time in the volume of frog muscles immersed in a 20 per cent urea solution.

A—dehydration of the muscles (in per cent of control); B—accumulation of urea in the muscles (in per cent per 100 g tissue water, concentration of the initial urea solution—4 per cent).

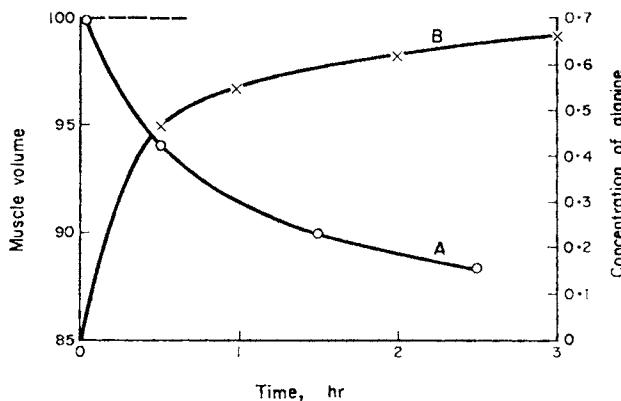


FIG. 18. The change with time in the volume of muscle in a 1.69 per cent solution of alanine.

A—dehydration of the muscle (as per cent of control); B—accumulation of alanine in the muscle (in per cent per 100 g tissue water, the concentration of the initial alanine solution being 2.0 per cent).

of the various substances in conditions of water equilibrium. The figures show the dehydration with time of frog muscles in solutions of urea (Fig. 17), alanine (Fig. 18), galactose (Fig. 19) and sucrose (Fig. 20) and the absorption by the muscles of these substances. The data relating to the distribution

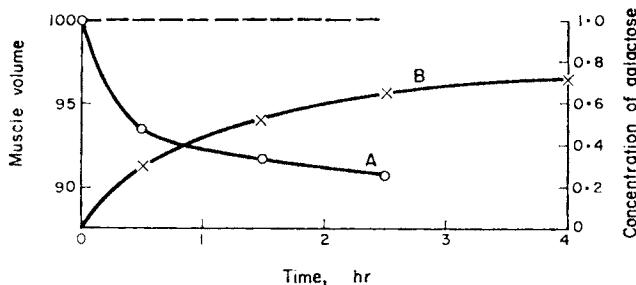


FIG. 19. The change with time in the volume of muscle in a 1.8 per cent solution of sucrose (after Nasonov and Aizenberg, 1937, and Kamnev, 1938).

A—dehydration of the muscle (as per cent of control); B—accumulation of galactose in the muscle (in per cent/100 g tissue water, the concentration of the initial galactose solution being 2.0 per cent).

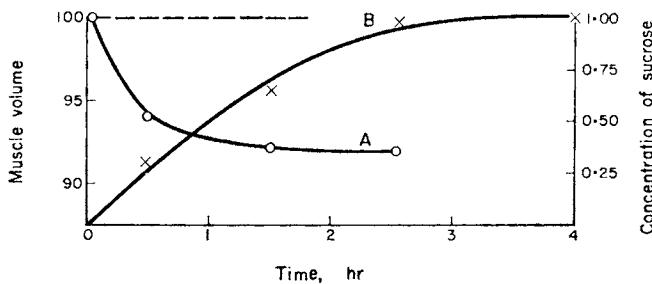


FIG. 20. The change with time in the volume of muscle in a 4 per cent solution of sucrose (after Nasonov and Aizenberg, 1937, and Kamnev, 1938).

A—dehydration of the muscles (as per cent of control); B—accumulation of sucrose in the muscle (in per cent/100 g tissue water, the initial sugar concentration being 4 per cent).

of urea and alanine were obtained by myself, while the curves for the accumulation of galactose and sucrose in the muscles are taken from Kamnev (1938).

The curves in these figures clearly indicate that the muscles lose water simultaneously with the permeation into them of the substances. It is noteworthy that water equilibrium is achieved at the same moment as diffusion equilibrium between the substance that has permeated the muscle fibres and

that which is in the external solution, whereas, according to the membrane theory, the volume of the muscles should return to its initial size towards this time.

The following figures show the dehydration of the muscles in solutions of urea (Fig. 21), alanine (Fig. 22), galactose (Fig. 23), and sucrose (Fig. 24) in relation to the concentration of these substances in the medium under conditions of water equilibrium. There is also shown in these figures the equilibrium concentration of the substances in the muscles for each corresponding concentration in the medium. The curves show that the greater the equilibrium concentration of a substance in the medium, the greater the degree

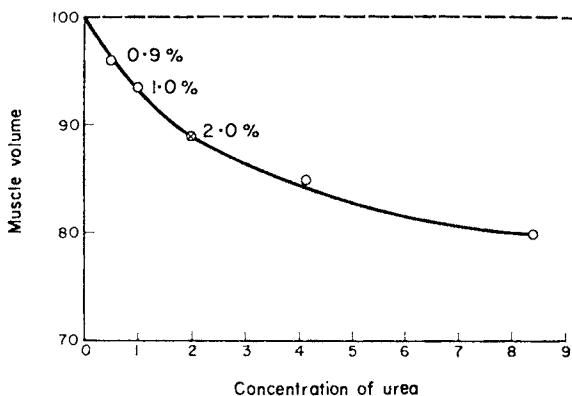


FIG. 21. The relation between the volume of frog muscle (expressed in per cent of control) and the urea concentration (in per cent) in the medium. The figures above the curve denote the equilibrated urea concentrations in the muscle fibres (the changes in muscle volume are taken from Nasonov and Aizenberg, 1937).

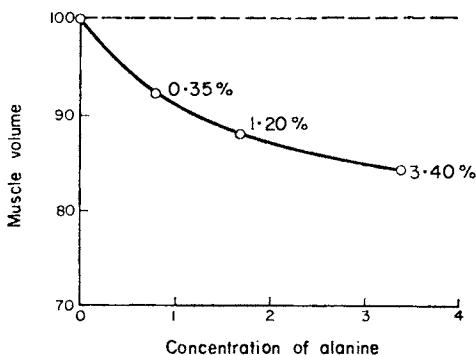


FIG. 22. The relation between the volume of frog muscles and the concentration of alanine in the medium.

(The changes in muscle volume are from Nasonov and Aizenberg, 1937.)

of dehydration of the muscles and the more of this substance there is in the muscles. It is interesting that this rule is applicable both in the case of the effect on the dehydration of the muscles due to urea, concerning whose permeation into the muscle fibres nobody was in any doubt, but also in the

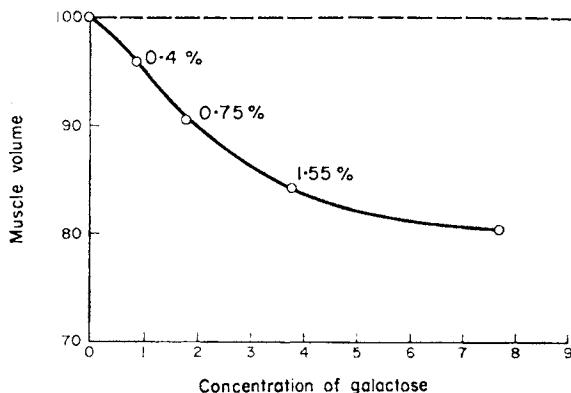


FIG. 23. The relation between the volume of frog muscles and the concentration of galactose in the medium. (The changes in muscle volume are from Nasonov and Aizenberg, 1937.)

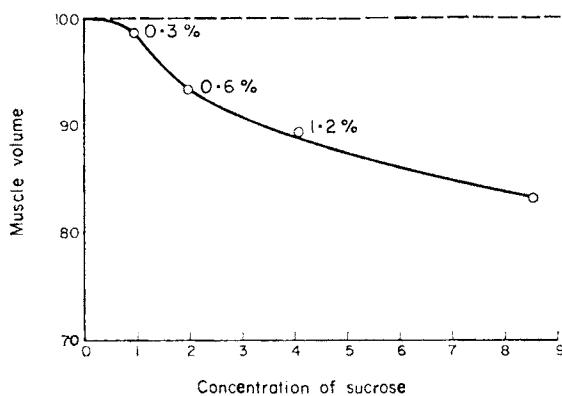


FIG. 24. The relation between the volume of frog muscle and the concentration of sucrose in the medium. The numbers above the points show the intracellular concentration. The unit of concentration is per cent. (The changes in muscle volume are from Nasonov and Aizenberg, 1937.)

case of the action of alanine and the sugars, which are supposed to penetrate muscle only with difficulty or not at all.

In solutions of strong electrolytes live cells likewise do not behave like osmometers. It is important to notice this, because it has been thought up to the present time that sodium chloride in balanced salt solutions and in

blood plasma essentially plays only an osmotic role and can be replaced by any neutral non-electrolyte, for example, sugar, which, just like sodium chloride, does not, from the point of view of the membrane theory, penetrate cells (Rubinshtein, 1947, p. 189).

There are many facts that can be adduced against this assertion. Thus, Steinbach (1944) studied the swelling and shrinking of frog muscles in Ringer's solutions, in which the concentration of sodium chloride was varied far from the normal both on the high and the low side. At the same time

TABLE 7. THE CHANGE IN THE QUANTITY OF WATER AND MINERAL SUBSTANCES  
IN FROG MUSCLE IN RELATION TO THE CHANGE IN THE CONCENTRATION  
OF SODIUM CHLORIDE IN RINGER'S SOLUTION  
(from Steinbach, 1944)

Chloride in the medium m eq. %	Final weight of the muscles as % of the initial weight	Concentration in the muscle (in m-equivalents per 100 gm of tissue)				
		K <sup>+</sup>	Na <sup>+</sup>	Cl <sup>-</sup>	Na <sup>+</sup> — Cl <sup>-</sup>	Na <sup>+</sup> + K <sup>+</sup>
17·0	90	9·60	7·87	6·26	1·61	17·48
12·0	98	8·93	3·75	3·20	0·65	12·86
9·5	107·5	8·90	2·75	2·67	0·07	11·65
7·0	135·0	7·47	1·57	1·82	— 0·27	9·04

that he determined the change in the water content of the muscles, Steinbach determined in the muscles the change of the amount of the fundamental "osmotically active" ions—potassium, sodium and chloride (Table 7). As a result of a careful analysis of the data obtained, he came to the conclusion that there is no connection between the change in the water content of the muscles and osmotic phenomena.

Thus, one must conclude that the laws that underlie the regulation of the water content of the protoplasm are not osmotic, but colloidal. This assertion is supported by the fact that live cells lose water in solutions of various substances at the same time as the latter penetrate the protoplasm. Under these conditions the mechanism for distributing water between the cells and the medium can only be colloidal. As its foundation are the hydration of the colloids and the change in this hydration as a result of the penetration of substances from without into the cells.

In the light of the experimental data set out in this chapter it is possible to find a rational explanation for the results of some experiments by Hill (1930) on the measurement of the vapour tension of solutions in equilibrium with the cells. It was on the basis of these experiments that Hill, as is well known, came to the conclusion that cells (muscle fibres and erythrocytes) contain practically no "bound" water: almost all the water, apart from 4–5 per cent, is in the protoplasm in the same state, as it were, as it is in Ringer's solution.

This conclusion of Hill's, as we have seen, contradicts an enormous quantity of facts.

Hill's experiments do not really indicate anything about the state of the water in the cells. What in fact happened was that Hill measured the fall in vapour tension of Ringer's solutions to which had been added a small quantity of some substance, for example, sugar. The fall in the vapour tension of this solution was compared with the fall in vapour tension of the same solution in which the cells had previously been placed. As we have seen, the change in the degree of hydration of the cell colloids for a small deviation from "isotony" of the surrounding medium occurs just as though the cells behaved like perfect osmometers under these circumstances. It is only when there is a more marked deviation from "isotony" that there is also a deviation from the law of osmosis. In Hill's experiments the substance added to the balanced salt solution in part penetrated the cells and a small amount was bound by the protoplasmic colloids, while the remainder in the external solution was diluted by the water emitted by the cells. Therefore what Hill measured in his experiments was not the amount of "bound" water in the protoplasm, but the degree of dilution of the external balanced solution because of the permeation of the added substance into the cells and the emission of water from them.

Had Hill added to his salt solutions greater quantities of the substance, he would have obtained a higher percentage of "bound" water in the cells, because in strongly hypertonic conditions, the cells lose considerably less water than they should according to the osmotic law, while, if he had added less, he would have obtained a quantity of "free" water in the cells greater than the total amount of water in the protoplasm, as he in fact did in his experiments with urea, which permeates cells in large amounts and is strongly adsorbed by the cell colloids.

Thus, the quantity of water in the protoplasm is regulated by the degree of hydration of the colloids of the live substance and not by the membrane mechanism.

In view of this the concepts which are embedded in physiology of "isotonic", "hypertonic" and "hypotonic" solutions do not reflect the essential nature of the phenomena and should, therefore, either be replaced by others or be given a new meaning.

"Isotonic solutions" are those in which the hydration of the protoplasmic colloids remains unaltered, as a result of which the water content of cells immersed in such solutions remains at its former level. In "hypotonic solutions" there is an increase in the hydration of the colloids of the live substance, so that cells in them gain water, while in "hypertonic solutions", on the other hand, there is a dehydration of the protoplasmic colloids, as a result of which the cells lose water and decrease in volume.

Of course, one should not deny the possibility of osmotic phenomena in cells in general and in all cases. The vacuoles of plant cells, as has been

mentioned above, behave in solutions of a number of substances like osmometers. In this case the movement of water into and out of the vacuole is accomplished by means of osmotic forces. This can happen because the diffusion of water through the surface layer of the protoplasm and the tonoplast takes place more quickly than the diffusion of solutes. But contraction of the vacuole (plasmolysis) and the consequent emission of water can occur also as a result of the diminution in volume of the cytoplasm caused by the penetration into the protoplasm of some substance, as happened in the experiments of Walter (1923) with non-vacuolar plant cells and in the experiments just described with animal cells.

### 3. *The Permeability of Cells to Water*

The change in volume of cells that occurs on their transfer from one medium to another clearly indicates that the cells are permeable to water molecules. A vast amount of information on the permeability of cells to water has been obtained by osmotic methods; but, as cells do not behave like osmometers, these data cannot be considered to be completely reliable. At least this refers to animal and non-vacuolar plant cells.

However, as has been shown in the preceding paragraph, in solutions of many substances, where the concentration does not differ greatly from normal ("isotonic") solutions, the curves for the swelling and contraction of the cell colloids behave as though the cells had the properties of perfect osmometers. Therefore many of the facts obtained by osmotic methods can give a more or less correct impression of the speed of penetration of water into cells and from cells into the surrounding medium. Such data, taken from the work of selected authors, are shown in Table 8.

The permeability of cells for water changes strongly, depending on their physiological state. Cells can absorb water in great quantities under the effect of certain enzymic poisons, low temperature and certain other factors and can again lose water on being returned to normal conditions (see p. 355 ff.).

It has been shown by many workers that fish egg cells change their permeability to water sharply after fertilisation or artificial activation. Further, Loeb (1894, Loeb and Wasteneys, 1915) has shown that the fertilised eggs of *Fundulus* develop normally in either much diluted or "hypertonic" water without any change in volume. Data obtained in experiments on trout eggs (Gray, 1920, 1932; Svetlov, 1929; Krogh and Ussing, 1937; Prescott and Zeuthen, 1953) and salmon eggs (Manery and Irving, 1935; Hayes and Armstrong, 1942; Hayes, 1949; Prescott, 1955, etc.) show that after fertilisation or artificial activation the egg cells become impermeable to water. In the opinion of several writers, this impermeability to water is achieved by a change in the properties of the plasma (yolk) membrane after fertilisation or activation of the egg, while the corium remains freely perme-

able to water (Gray, 1920, 1932; Svetlov, 1929; Yagle, 1930; Prescott, 1955, etc.).

Recently, Kusa (1951) and Kanoh (1952) have shown that the plasma (yolk) membrane of unfertilised salmon eggs is permeable to water. The same conclusion is reached by workers who have studied this problem in experiments with heavy water (Krogh and Ussing, 1937; Ussing, 1952; Prescott and Zeuthen, 1953; Prescott, 1955). They found that heavy water passes slowly through the plasma membrane of unfertilised eggs, but that after fertilisation the membrane allowed absolutely no water to pass through it. This change in the properties of the yolk membrane after activation of the

TABLE 8. THE PERMEABILITY OF CELLS TO WATER FROM THE DATA  
OF OSMOTIC EXPERIMENTS

The permeability constants  $P$  are given in  $\mu^3$  per min through  $1 \mu^2$  of surface at a difference of osmotic pressure inside and outside the cell of 1 atmosphere.

	$P$	Authors
<i>Protozoan cells</i>		
Amoeba proteus	0.026-0.031	Mast and Fowler, 1935
Pelomyxa carolinensis	0.023	Belda, 1943
Zoothamnium	0.12-0.25	Kitching, 1938
Gugarina	0.2	Adcock, 1940
<i>Unfertilised eggs of marine animals</i>		
Arcacia punctulata	0.10-0.17	
Chaetopterus pergamentaceus	0.44-0.46	
Cumingia tellenoides	0.41-0.46	Lucké <i>et al.</i> , 1935, 1939,
Ostrea virginica	0.60-0.70	1941
Dendraster excentricus	0.20-0.27	
Palizia miniata	0.39-0.44	Leitch, 1931
Strongilocentrotus franciscanus	0.11-0.14	
Strongilocentrotus purpuratus	0.09-0.10	
<i>Plant cells</i>		
Tolypellosis stelligera	1.08	Palva, 1939
Fucus vesiculosus	0.13-0.19	Resühr, 1935
Allium cepa	0.35	Levitt <i>et al.</i> , 1936
Salvinia auriculata	0.55	Huber and Höfler, 1930
Majanthemum bifolium	0.06-0.38	
Convallaria majalis	0.33-0.40	Seemann, 1950a, 1950b
Salvinia natans	0.37-0.51	
<i>Mammalian cells</i>		
Mouse, rat and chicken fibroblasts	0.4-1.0	Brues and Masters, 1936
Rabbit leucocytes	0.3 }	Shapiro and Parpart,
Human leucocytes	1.3 }	1937
Bovine erythrocytes	2.5	
Human erythrocytes	3.0	Jacobs, 1932

egg is explained by the adsorption onto it of lipoproteins (Ussing, 1952; Prescott and Zeuthen, 1953; Prescott, 1955).

Recently, however, Buno and others (Buno and Chambers, 1947; Chambers, Chambers and Kao, 1951; Kao and Chambers, 1954; Kao, Chambers and Chambers, 1951, 1954), studying the internal hydrostatic pressure of *Fundulus* eggs under various experimental conditions, came to the conclusion that the plasmatic (yolk) membrane is permeable for water both before and after fertilisation or activation of the egg.

In this connection the meticulous work done by Stroganov (1938) with perch roe is of great interest. He showed that the volume of the actual egg (nucleus and yolk), whether fertilised or unfertilised, when placed in water, first increases, then decreases, and then increases again. There were particularly large variations in the volume of the fertilised egg cells.

The use of heavy water ( $D_2O$ ) and radioactive ( $T_2O$ ) water for studying the speed of penetration of water into cells and the replacement of cell water by water from the surrounding medium does not always lead to identical results. Thus, on the introduction into the blood stream of rabbits of heavy water, it mixes in as little as half a minute with all the extra-cellular water and in 30 to 40 min with all the body water. However, after 39 days one fifth of the heavy water introduced into the blood is still left in the body of the animal (Hevesy and Jacobsen, 1940; Hahn and Hevesy, 1941a). It was observed by Kutyurin (1956) that the cell water of water thyme is half replaced by heavy water from the medium in the space of an hour, but that the remaining water is not replaced for a long time. Water labelled with tritium was found to behave similarly in experiments with plant (Cline, 1953) and animal (Tompson and Ballou, 1953) cells.

Prescott and Mazia (1954) showed that 81–92 per cent of the water of the protoplasm of *Amoeba proteus* as a whole and of the cytoplasm and nucleus is replaced by the heavy water of the medium. More than 99 per cent of the water is replaced by the heavy water of the medium in the first 14 min. Heavy water penetrates the giant amoeba *Chaos chaos* at the same speed (Pigon and Zeuthen, 1951).

The permeability of erythrocytes to heavy water was studied by Brooks (1935a, b) and Parpart (1935). According to the latter, heavy water penetrates cattle and rat erythrocytes considerably more slowly than normal water. However, Lucké and Harvey (1935) observed  $D_2O$  and  $H_2O$  to pass into the egg cells of sea urchins with equal speed.

A detailed description of work on water exchange in fish and cyclostomata embryos is given in a monograph by Zotin (1961), who considers the water exchange of cells as "an active process".

The permeability to water of the envelope of the cell nuclei and of the tonoplast of the vacuoles of plant cells is, apparently, as high as for the cells themselves (Huber and Höfler, 1930; Beck and Shapiro, 1936; Höfler, 1950, etc.).

*4. Conclusions*

1. Animal and non-vacuolar plant cells do not obey the law of osmosis.
2. The assumption that there is a definite amount of "bound water" cannot explain the deviations from the osmotic law in the change of volume of cells, because the correction for the osmotically inert volume (the bound water) for different substances that are assumed not to penetrate the cell varies with the substance. Further, in solutions of substances with large molecules the cells lose significantly more water than the law of osmosis requires.
3. The increase or decrease in the water content of a cell in solutions of different substances is a colloidal phenomenon connected with the penetration of molecules of the solute into the cell.
4. In view of what has been said in the first three paragraphs above, the so-called "osmotic behaviour" of cells cannot be used as a proof of the existence of a semi-permeable membrane regulating, as is supposed, the entry of substances into the cell.
5. The change in volume of vacuolar plant cells obeys the law of osmosis. In this case, the role of the semi-permeable membrane is, apparently, played by the tonoplast.
6. Cells are freely permeable to water. Not all the water of the cell is replaced with equal speed by water from the surrounding medium that has been labelled with deuterium or tritium.

## CHAPTER III

# The Protoplasm as a Colloidal System

### 1. *Introductory Remarks*

As we have already mentioned, there was another theory brought out in opposition to the membrane conception of cell permeability that attempted to explain the phenomena of cell permeability and the characteristics of the distribution of substances between the cell and the medium in terms of the laws of sorption.

The sorptional theory of cell permeability has been developed most fully by Nasonov and his colleagues (Nasonov and Aleksandrov, 1934, 1937, 1940, 1943, 1945; Kamnev, 1938; Nasonov, 1939, 1959; Aleksandrov, 1939a; Troshin, 1939, 1953a).

From the standpoint of this theory, the greater or lesser permeability of a cell to some substance is determined by the sorptional level of the protoplasm as a whole (solubility, adsorption and chemical binding). The higher the sorptional level of a cell in any given functional state in relation to some substance, the greater the amount of that substance that will penetrate the cell, and, on the other hand, the lower the sorptional level, the less pronounced will the permeability of the cell to that substance be.

This theory starts from the point that the dispersion medium of the protoplasm (water) is in a special state and behaves in relation to the aqueous solution of substances surrounding the cell like a non-aqueous phase: the solubility in it of the various chemical compounds is significantly different from their solubility in the water of the surrounding medium.

An argument often brought forward against this theory is that there is in the protoplasm a very great quantity of water, several times greater in weight than the dry residue, and that this water cannot differ in its properties from ordinary water (Hill, 1935; Rubinshtein, 1939, 1947, 1949a, b, etc.).

However, it is now well known that proteins, lipoids, nucleic acids and many other substances are not independently soluble in the water of the cell but form intricate complexes. This last circumstance can make a radical difference to the state and properties of the water that forms part of the protoplasm in comparison with its properties in a normal aqueous solution.

The suggestion has frequently been made that the protoplasm has a coacervate structure and that the water in it is somehow organised, "bound" by hydrophilic substances in the same way as it is "bound" in coacervates.

In fact coacervates are very reminiscent of the protoplasm in a number of their physico-chemical properties, and it is on this basis that many workers have made the suggestion that it is coacervates that play the fundamental role in the structure of the protoplasm (Bungenberg de Jong, 1932; Duclaux, 1934; Guilliermond, 1941; Oparin, 1941, 1954, 1957; Makarov, 1948a, 1950; Danzhar, 1950; Troshin, 1953; Oparin and Yevreinova, 1954; Yevreinova, 1954, 1956; Yevreinova and Shubert, 1955; Yevreinova, Korolev and Agroskin, 1959; Yevreinova and Kuznetsova, 1959; Segal, 1958; Linke, 1959; Nasonov, 1959a, etc.).

Oparin (1941, 1945, 1954, 1957) supposes that the protoplasm of the cells of organisms alive to-day is a complex coacervate. In his opinion, it was from particles of dead matter that drops of coacervates first arose, from which, at a later date, the first organisms developed.

It should, however, be remarked that the recognition of the protoplasm as having a coacervate nature is not the only possible way of overcoming the difficulties of explaining the phenomena of cell permeability. Lepeschkin (1930, 1939), Fischer and Suer (1935, 1938, 1939), Frey-Wyssling (1950), Ling (1952, 1955, 1957, 1958, 1960), Shaw and Simon (1955b), Szent-Györgyi and his colleagues (Szent Györgyi, 1957; Baird, Karreman, Mueller and Szent-Györgyi, 1957), Ungar (1959) and others have suggested various concepts of the submicroscopic structure of live matter which get round the difficulties met by the membrane theory.

## 2. Coacervates and their Formation

The sol of any hydrophilic colloid can, under certain conditions, pass into such a state that its disperse phase separates from the dispersion medium, not in the form of a solid precipitate, as happens on coagulation, but rather as a drop or liquid layer which contains a considerable amount of the solvent and does not mix with it. What happens is that the colloidal particles which were previously distributed uniformly in the solution are, as it were, collected in one place. A cluster of colloidal particles is formed; they are all piled up together. It is for this reason that the process of separating off and concentrating the sol was called "coacervation" (from the Latin *acervus* — a heap or pile).

The characteristic features of a coacervate are that it contains a considerable amount of the solvent (dispersion medium), sometimes as much as 4–5 times more than the disperse phase, and that it is immiscible with the liquid in which it arose and with which it is in equilibrium. A third important feature is that the colloidal particles in the coacervate interact in such a way that their individual properties are preserved, a circumstance which ensures that the coacervates can react with other solutes entering them and also gives them their fluid properties (Bungenberg de Jong and Kruyt, 1930).

Initially Bungenberg de Jong and Kruyt (Bungenberg de Jong and Kruyt, 1930; Bungenberg de Jong, 1932, 1937a, b, etc.) supposed that the coacervates are formed as a result of the disruption of the diffusion layer of the hydrated envelopes of the colloidal particles. The hydrated layer that remains, so it could be thought, consists of strictly oriented water molecules with a sharp boundary separating this aqueous layer from the water of the surrounding medium. Such colloidal particles combine to form a common solvate envelope

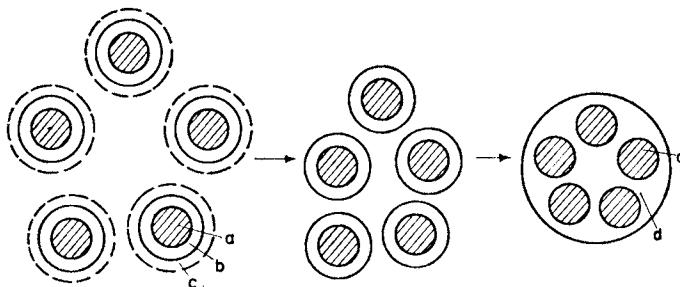


FIG. 25. Scheme of the formation of coacervate drops (from Bungenberg de Jong and Kruyt, 1930).

a—colloidal particle; b—c—solvate envelope (b—solid layer, c—diffusion layer); d—disperse medium of the coacervate-solvate liquid.

of strictly oriented water molecules. The particles do not, however, combine completely inside such a droplet because of the resistance of the internal layers of the hydrophilic envelopes of the particles. The coacervate drops in combining with one another become separated from the bulk liquid with which it is in equilibrium to form a layer. This mechanism for the formation of coacervates is shown in Fig. 25.

This theory of coacervates of Bungenberg de Jong's was criticised by Mikhailov (1935), Lepeschkin, Dervichian (1949) and others.

Lepeschkin (1939) showed that the de-solvation of the colloidal particles on the formation of coacervates is not partial but complete; it is only after this that the colloidal particles can form a complex—a porous chemical combination. In his opinion, the water in these complex formations saturates the micelles of the colloid and, consequently, is itself in the dissolved state. He therefore suggests that the term "coacervates" should be rejected as not reflecting the true nature of the phenomenon and should be replaced by his term "fluoid". A similar point of view on the nature of coacervates is expressed by Mikhailov in his book (1935).

To-day Bungenberg de Jong and his colleagues have dropped their initial theory of coacervation. One of their reasons is that the amount of "bound water" that the old theory of coacervation required could not be found experimentally in solutions of biocolloids. Some writers, however, hold another view, namely, that the water of the coacervate is solvent water and that

the amount in the coacervate corresponds to the amount of "bound water" (Bladernen, 1951; Oparin, 1957; Nasonov, 1959a).

At present Bungenberg de Jong (see Booij and Bungenberg de Jong, 1956) explains the phenomenon of coacervation in terms of phase theory. We know to-day of a great number of pairs of liquids with limited mutual solubility. Under certain conditions, the mixing of such liquids leads to their being dissolved in one another and forming a single phase system. If these conditions are changed, then the solubility of the molecules of the one liquid in the other may be reduced, as a result of which one liquid is separated from the other—the single phase system becomes biphasic. For example, phenol dissolved in water at 70° in the proportion 1 : 1 forms a single phase system with the water. If the temperature is reduced to 60°, the solubility of the phenol is reduced and the phenol comes out of solution so that the single phase system becomes biphasic. This example can be considered as a simple case of coacervation.

It was shown earlier that solutions of organic substances of high molecular weight, including protein solutions, behave like thermodynamically stable molecular solutions obeying the phase law (Tager, 1951). The applicability of the phase law to solutions of gelatine and many other substances of high molecular weight was shown by Kargin and his colleagues (1937a, b, c, 1939) and Tager and Kargin (1941).

The sol of a hydrophilic colloid (one phase system) can form a biphasic system under the influence of various factors that lower the solubility of the colloid. The solubility of the colloid can be lowered by a change in temperature or pH, the addition to the sol of various substances, and so on. The colloid phase can become separated in the form of coacervates (liquid phase), colloidal crystals (solid phase), flakes or individual colloidal systems.

The molecules of substances of large molecular size, such as proteins, behave like colloidal particles when dissolved in water. In solutions they form more or less solid clumps. The addition to such a solution of a substance of low molecular weight may reduce the solubility of the large molecules (colloidal particles) by reducing the affinity of the various hydrophilic groups along the length of the rolled-up macromolecular chain to water molecules. In consequence, a considerable amount of the adsorbed water within the molecule (colloidal particle) is lost. This leads to the association of the colloidal particles and their separation into a separate phase (coacervation). The new scheme of coacervation suggested by Bungenberg de Jong is shown in Fig. 26. The points of contact of the macromolecules in the coacervates are dynamic in character—these are loose combinations of colloidal particles, because a coacervate is a typical liquid with fairly high viscosity (Booij and Bungenberg de Jong, 1956). It should be noted that this concept of the mechanism of coacervation had already been put forward twenty years ago by Lepeschkin (1939).

To-day, simple coacervates, those which form in the sol without the inter-

action of ionised groups of colloidal particles (for example, gelatine with sodium sulphate, alcohol or resorcinol), are distinguished from complex coacervates, which arise owing to the interaction of oppositely charged groups of colloidal particles with the formation of a salt-like compound (for example, a solution of gelatine with a solution of gum arabic at a pH lower than 4·8, in which case the gelatine is positively charged and the gum arabic negatively [Booij and Bungenberg de Jong, 1956]).

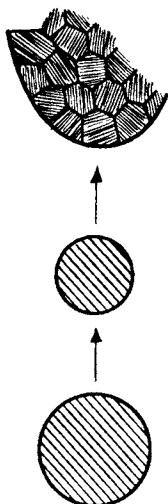


FIG. 26. Modified scheme of coacervation (from Booij and Bungenberg de Jong, 1956).

It is to this type of colloidal system that tactoids belong—coacervates of fibrillar proteins with a fusiform molecular shape. They consist of elongated molecules placed in line. Tactoids play an important part in the formation of the various structures of the protoplasm. They are formed on the partial dehydration of the fibrillar colloid or on the partial neutralisation of its charges. In these conditions the less hydrated (less soluble) particles of the colloid mix with the larger structures which contain less solvent (Frey-Wyssling, 1950).

Associative colloids obviously play an important part in the structure of the protoplasm. In this case the colloidal particle (micelle) is formed from the grouping of small molecules or ions, which do not behave like colloidal particles in an ordinary solution. Under certain conditions such colloidal associations are formed by such substances as phosphatides, various salts of fatty acids and alkali metals (for example, oleate or sodium stearate), etc. The molecules of these substances, which are joined by their hydrophobic ends, can form micelles of spherical and cylindrical shape or films two molecules thick.

In all these formations the molecules are strictly oriented with their ionised ends pointing outwards and their hydrophobic ends inwards. Such micelles can be in the form of a sandwich. The coacervates formed of such micelles are not amorphous, but reveal a definite regularity, a crystal structure. They have structural strength and elasticity. Protein molecules can join on to the surface of such micelles, rods and films. This type of complex formation of associative colloids is supposed by Bungenberg de Jong and his colleagues to take part in the structure of the cell's external membrane and in the structure of the various internal membranes and other structures (Booij, 1954, 1960; Booij and Bungenberg de Jong, 1956).

According to Lepeschkin (1936, 1939) the protoplasm is a special sort of fluid—a complex formation of proteins with lipids, which he calls a lipo-proteid or vitaid. Water and many other substances are dissolved in these vitaids. Lepeschkin's hypothesis encountered a number of difficulties and was severely criticised (Kizel', 1940; Nasonov and Aleksandrov, 1940; Makarov, 1948a, etc.).

A different view of the structure of the protoplasm was suggested by Fischer and Suer (1935, 1938, 1939). In their opinion, living matter consists of giant protein molecules with acidic and basic properties. These molecules are associated with water, mineral ions and other substances. The model they suggested for this hypothetical combination is the liquid obtained by mixing solutions of sodium caseinate and caseine chloride: this, as can be seen from a description of its properties, bears all the marks of a coacervate.

Arguing against the identification of the cytoplasm and nucleus with coacervates, Frey-Wyssling (1950) asserts that coacervate drops are completely amorphous, whereas the cytoplasm and nucleus have a definite structure. "The structure of the protoplasm", he writes, "is formed by the interweaving of the threads or rods of stromatin, which make up the molecular skeleton to which the lipoids, phosphatides, sterols, inorganic ions and water molecules are joined" (p. 169). According to Frey-Wyssling, this structure can easily be broken up, whereupon the cytoplasm becomes a typical liquid. As we shall see below, such a conception of the submicroscopic structure of live matter does not contradict the hypothesis of the coacervate nature of the protoplasm, because structures of a similar nature can occur in coacervates too.

With the great diversity of substances of which the protoplasm consists and their sharply individual characteristics there is ample opportunity for the formation of complex coacervates and the formation within them of various structures, complex formations of granular, fibrillar, membrane and other shapes. According to Bungenberg de Jong (1932, 1936a, b) there may be included in the structure of the protoplasm coacervates resulting from the interaction of protein with protein, of protein with lipids, of protein with nucleic acids, of protein with polymeric carbohydrate and so on.

### 3. Some Biologically Important Properties of Coacervates

As has already been remarked, many of the physico-chemical properties of coacervates resemble very closely some of the properties of the protoplasm. We indicate here only some of the common features in the behaviour of the two formations.

*Immiscibility with the surrounding medium.* Coacervates are liquid drops rich in colloids and containing from 50 to 85 per cent water. They are immiscible with the surrounding liquid, which is almost completely free from colloids. The protoplasm of animal and plant cells is likewise rich in water and immiscible with the surrounding aqueous medium. It is well known that if the envelope is broken, the protoplasm of animal and plant cells can be forced out of the cell into the surrounding aqueous medium, where it is split up into a multitude of drops which are immiscible with water and retain a number of the characteristic properties of the protoplasm in its undamaged state.

This phenomenon has been known since the time of Nägeli (1855), who made observations on plant cells (on the crushing of the root fibres of *Hydrocharis*). A similar phenomenon was observed by Kühne (1864) in the cells of protozoa and by Pfeffer (1877) in plant cells. The phenomenon was later studied in detail by Heilbrunn (1928) in experiments with the eggs of *echinoidea* and certain worms and by Lepeschkin (1924, 1925, 1926b, 1937, 1939) in a wide variety of animal and plant cells. The latter (Lepeschkin, 1939) studied the behaviour of protoplasm drops crushed into a sugar solution from the giant cells *Chara fragilis* and from the eggs of several kinds of *echinoidea*. Part of the numerous protoplasm drops coagulated, but part behaved just like the whole protoplasm, being immiscible with water. As a rule, the formation of Heilbrunn's envelopes was not seen. Consequently, the protoplasm is immiscible with the surrounding liquid even in the absence of an envelope.

Protoplasm drops can merge with one another, like coacervate drops. In either case vacuoles form easily.

On the plasmolysis of plant cells, the surface layer of the protoplasm breaks away from the main mass of the cytoplasm (Sabinin, 1940); nonetheless, the protoplasm of plasmolysed cells is also insoluble in the water that penetrates the envelope but remains sharply divided from it.

Seifriz (1936, 1938, 1941, 1947) also produces a large amount of convincing evidence for the protoplasm being a liquid immiscible with the water of the surrounding medium.

Thus, this feature of the protoplasm reminds one of coacervates which are also immiscible with their surrounding liquid.

*Vacuolisation.* There is a great variety of influences (acids, salts, bases, saturated and unsaturated hydrocarbons, alcohols, aldehydes, ketones, ethers, change in temperature, action of electric current, etc.) which can give rise to the formation in coacervates of multitudinous vacuoles which can

merge into one large vacuole. In some cases the multitudinous vacuoles fill up the whole of the coacervate drop, giving it a foam-like appearance. When the cause of the vacuolisation of the coacervate is removed, the vacuoles disappear and the coacervates again look the same as before.

The formation of vacuoles in a coacervate indicates that in this case the coacervate complex becomes less hydrated (Bungenberg de Jong, 1937a, b, 1947).

The formation of vacuoles in the protoplasm can also be caused by a wide variety of influences in widely differing plant and animal cells. Thus, vacuolisation of the protoplasm in protozoa and in the cells of multicellular plants and animals has been observed when there has been an increase in temperature, mechanical attack (pressure, pricking, etc.), irradiation with ultra-violet light, X-rays and radium rays, treatment with various drugs, acids and bases, or passage through the protoplasm of a direct electric current. Vacuolisation of the protoplasm is also observed under the effect of very small concentrations of the salts of heavy metals.

Live matter has frequently been observed to vacuolise under the action of the salts of the alkali and alkaline earth metals and also in hypotonic salt solutions and other reagents (see the references in Lepeschkin, 1924, 1937; Heilbrunn, 1928; Nasonov and Aleksandrov, 1940; Guilliermond, 1941 and Makarov, 1948a). Various agents have been observed to cause the vacuolisation not only of the protoplasm, but also of the nucleus, nucleolus, chondriosome and other organoids of the cell (Heilbrunn, 1928; Guilliermond, 1941, etc.). This indicates that the organoids of the cell, just like the cytoplasm, are coacervate in nature (Duclaux, 1934; Bungenberg de Jong, 1936a, b; Guilliermond, 1941; Makarov, 1948a, 1950, etc.).

*The mechanism of the formation of structures in coacervates and in protoplasm.* Pictures of the formation in homogeneous coacervate drops of structures of various kinds are of great biological importance. The mechanism of their formation can shed a certain amount of light on the process of growth of the cell structures that are always present in the protoplasm (cell organoids) and which appear under definite physiological conditions and at definite stages of cell development.

Coacervate drops easily gelatinise if they contain colloidal particles which can, under certain conditions, form a gel from the state of a sol. Coacervate drops consisting of gelatine and gum arabic or gelatine and lecithin, clupeine, nucleic acids, etc., easily gelatinise upon a fall in temperature. The coacervate then often acquires a honeycomb structure, in which numerous vacuoles and structures of rod form, etc., can be seen. This can occur under the influence of a great range of physical and chemical agents.

Coacervate drops can easily become "liquid" and "solid crystals", forming so-called micellar crystals (Bungenberg de Jong, 1934, 1935). According to Bungenberg de Jong, the micelles (colloidal particles) in a drop of a complex coacervate are not strictly oriented one to another. However, if the

positive and negative charges on the micelles of a coacervate can somehow be increased (by change in pH, concentration of salts, etc.) or their degree of hydration be reduced (by the action on the coacervate of alcohols, tannin and certain other substances which can lower the degree of hydration of colloidal particles), then the micelles are brought closer together and oriented with respect to one another in a definite order (oriented coacervates), the coacervate drops becoming ellipsoidal in shape (if the micelles were previously sticklike) with the additional possibility of forming micellar crystals, fibrils or flakes of amorphous or fibrillar structure. Such changes of coacervates into complex "oriented coacervates", "micellar crystals" and fibrils and their reversion to coacervate drops have been studied thoroughly by Bungenberg de Jong and his colleagues for coacervates of various proteins, lecithin, nucleic acids, polymeric carbohydrates, etc. (Bungenberg de Jong, 1934; Bungenberg de Jong and Sengers, 1934; Bungenberg de Jong, Dekker and Winkler, 1934; Bungenberg de Jong and Hartkamp, 1934; Bungenberg de Jong and van der Linde, 1934a, b; Bungenberg de Jong, van der Linde and Dekker, 1935; Bungenberg de Jong, van der Linde and de Haan, 1935; Bungenberg de Jong and Bank, 1939a, b, c, 1940).

In the cytoplasm and nucleus of plant and animal cells we often observe structures which by the mechanism of their formation and their composition are in all probability formations of a coacervate nature—complex cell structures (myofibrils and neurofibrils, nuclear spindles at mitosis, microsomes, chondriosomes, chloroplasts, chromosomes, etc.). Actomyosin is, to all appearances, a typical complex formation of fibrillar structure. The development of chondriosomes and their transformation also points to their coacervate nature (Guilliermond, 1941).

According to Sveshnikova (1952), microsomes are intricate complex formations of a coacervate nature.

Another subject of great interest are the so called multiple complex and autocomplex coacervates and their transformations. The mechanism of their formation and their behaviour in various conditions can supply much information for the understanding of the physico-chemical organisation of the cell.

Complex and autocomplex coacervates, which consist of three or more components, can make up either one complex coacervate or a mixture of coacervates immiscible with one another, depending on the pH, the ratio of the amounts of one or other colloid present, etc. Thus, the complex coacervate: gelatine (+) + isinglass (+) + gum arabic (-) in every proportion of these substances forms only one complex coacervate. Coacervate drops of gelatine and gum arabic and of isinglass and gum arabic are miscible in all proportions. But the complex coacervate made up of gelatine, gum arabic and sodium nucleinate can exist as a complex coacervate or form two different complex coacervates immiscible with one another—drops of the one coacervate can contain drops of the other. If such a mixture is centrifuged,

it is separated into two layers: the heavy coacervate of gelatine and sodium nucleinate is left on the bottom of the vessel with the gelatine and gum arabic coacervate on top. Thus, if 2 per cent solutions of gelatine, gum arabic and sodium nucleinate are taken in various quantities and mixed at a pH of 3·6, drops of two coacervates are obtained, while if the ratio of the three components or the pH value is changed, one complex coacervate made up of three substances is obtained.

Colouring is a good way of observing double coacervates. For example, a drop of the complex coacervate formed by gelatine and sodium nucleinate inside a drop of gelatine and gum arabic coacervate is selectively coloured by methylene green (Bungenberg de Jong and de Haan, 1933).

The living cell is also, apparently, a system of multiple coacervates. The cell nucleus is a coacervate within another coacervate—the cytoplasm. The nucleolus in its turn is also a formation of coacervate nature (Bungenberg de Jong, 1932; Duclaux, 1934; Bungenberg de Jong and Bank, 1939a; Bungenberg de Jong, Bank and Hoskam, 1940; Guilliermond, 1941, etc.). A typical example of coacervate droplets within a coacervate is the process of the formation of granules of vital dyes in the protoplasm (Fel'dman, 1948a, 1950, 1953). The mechanism of the formation of granules of secretion in glandular cells evidently has much in common with the mechanism of the formation of multiple coacervates.

*The behaviour of coacervate drops in an electric field.* Simple coacervates are not affected by electric current, but complex coacervates are extremely sensitive to both alternating and direct current.

The picture of the changes in coacervates is in every detail strikingly like that of the protoplasm of live cells in similar circumstances.

When direct current is passed through a suspension of complex coacervate drops in an equilibrated liquid, the drops move either to the anode or to the cathode or remain stationary, depending entirely on whether the electric charges on the coacervate drops are balanced or not. If, in a mixture of two oppositely charged sols, there is an excess of positively charged colloidal particles over particles with negative charges, the coacervate drops will be positively charged, and vice versa. If the positive and negative charges in this mixture of sols are balanced, then the coacervate drop as a whole will not be charged. In the first case the coacervate drops on electrophoresis move towards the cathode, in the second towards the anode, and in the third remain stationary. Thus, if equal parts of 0·67 per cent gelatine solution and 0·67 per cent gum arabic solution are mixed at pH = 3·46, the coacervate drops as a whole will be uncharged; if more gelatine is used, the drops will be positively charged, if more gum arabic, negatively. Change of pH in either direction will produce a change in the charges on the drops.

This phenomenon has been studied in detail by Bungenberg de Jong and his colleagues (Bungenberg de Jong and Dekker, 1930, 1935, 1936; Bungenberg de Jong, Dekker and Gwan, 1930; Bungenberg de Jong and Gwan,

1930; Bungenberg de Jong and Kaas, 1931; Bungenberg de Jong and Lens, 1931; Bungenberg de Jong and Winkler, 1932).

Likewise, on the cataphoresis of a suspension of live cells, the movement is observed of some towards the cathode and others towards the anode (more frequently towards the latter). The contents of the cell (organoids, inclusions) are also displaced towards one or other pole if these formations are charged.

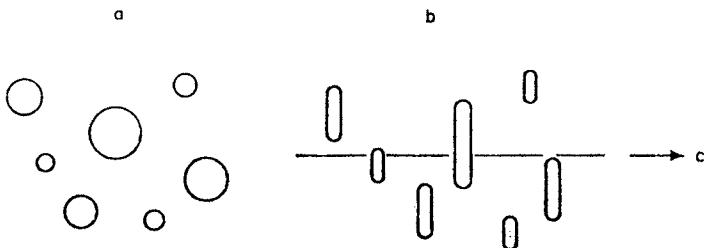


FIG. 27. Change in shape of coacervate drops on the passage through a suspension of them of an electric current (from Bungenberg de Jong and Dekker, 1930).

a—drops of coacervate in the normal state; b—coacervate drops under the action of an electric current; c—direction of current.

This phenomenon has been studied in a great number of different subjects—erythrocytes, yeast cells, bacteria, protozoa, spermatozoids, the cytoplasm of plant cells, etc. (Heilbrunn, 1928; Milovanov, 1935; Rubinstein, 1947; Shreder, 1949, etc.).

When a direct or alternating electric current is passed through a suspension of coacervate drops, they change into disks and become oriented perpendicular to the direction of the electric current (Fig. 27). This phenomenon indicates that the electrical conductivity of the coacervate is less than that of the equilibrated surrounding liquid (Bungenberg de Jong and Dekker, 1930).

When a direct current acts on a suspension of drops of a complex coacervate, disintegration occurs just as in the action of direct current on live cells. If an electric current of 20–60 V is passed through a suspension of drops of a coacervate made up of gelatine and gum arabic and positively charged, the coacervate drops slowly move towards the cathode, while inside them appear little vacuoles which rotate. These vacuoles merge into one, which then moves towards the surface of the coacervate drop, emerges on the side pointing towards the cathode and is dissolved in the equilibrating liquid. On the opposite side in the liquid numerous tiny coacervate droplets form afresh. After the emergence of the large vacuole from the coacervate drop the whole process starts again. If the coacervate drops are negatively charged, then the vacuole formed moves towards the anode and emerges from the drop on that side, while the corona of newly formed coacervate droplets is formed on the opposite side of the drop. The disintegration of the coacervate drops is shown schematically in Fig. 28. If, however, the electrical

voltage is sufficiently high, for example, greater than 100 V, then the coacervate drops are quickly changed into disks and disintegrate into a multitude of tiny droplets.

Similarly, in the protoplasm direct current is observed to produce vacuolisation and disintegration into fine droplets, which, at any rate in their outward appearance, recall the process of disintegration of coacervate drops. Disintegration of the protoplasm can occur both on the anode side (*Actinosphaerium eichhorni*), and on the cathode side (*Opalina ranarum*), and in protozoa on both sides (Heilbrunn, 1928).

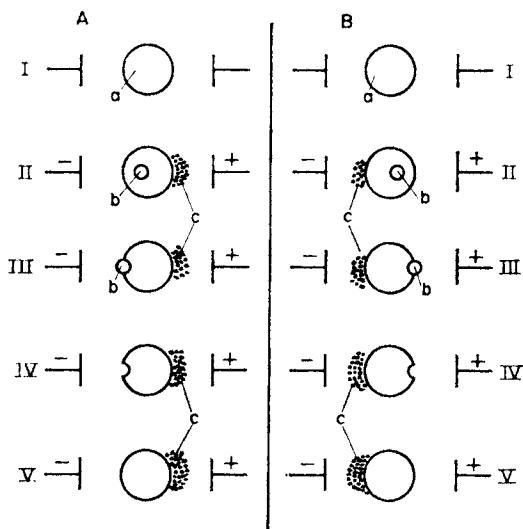


FIG. 28. Scheme of the disintegration of drops of a complex coacervate in a direct current electric field (from Bungenberg de Jong and Dekker, 1930).

I-V—stages in the disintegration of the coacervate; A—positively charged coacervate drop; B—negatively charged coacervate drop; a—drop of coacervate; b—vacuole; c—crown of newly formed coacervate droplets.

*Sensitivity to neutral salts, change in pH, temperature and other factors.* Simple coacervates are not very sensitive to the action of strong electrolytes or to changes in the concentration of hydrogen ions. On the other hand, complex coacervates show extreme sensitivity to these factors. In many respects the effects of various agents on coacervates recall their effect on the protoplasm. The sensitivity of complex coacervates to changes in pH and salt concentration is explained by the fact that these change the electrical charge on the colloidal particles that make up the complex coacervate. As a rule, neutral salts have the effect of diluting a complex coacervate; if there is a sufficient concentration of salts, the coacervate can decompose into two sols, from which other complex coacervates can be formed.

Many coacervates (for example, of gelatin and lecithin or gelatin and sodium nucleinate) form at a definite pH, at first in flakes. To change the flakes into drops, all that is needed is to add some electrolyte to reduce the forces of attraction (electrically opposite charges) on the colloidal particles of the coacervate.

Acids and alkalis can have one of two effects on complex coacervates. Depending on whether the attractive forces are increased or reduced, the coacervate may be diluted and decompose or, at certain concentrations, it can become more dense. If the charge on both the colloids is increased, the coacervate will suffer an increase in density, whereas if there is an increase in charge on only one while the charge on the other falls sharply, the coacervates will be diluted and will decompose. If an electrolyte, acid or alkali has the effect of compressing (increasing the density of) the coacervate, then vacuoles are formed. On the other hand, if the attractive forces of the complex are reduced, the vacuoles already in the coacervate will disappear and the coacervate itself be diluted.

Neutral salts, acids and alkalis can produce the above mentioned effects on any complex coacervates. The effect of the salts on complex coacervates increases with increasing valency of the anion and cation—in accordance with Hardy's law.

Gelatinised drops of coacervate, when attacked by alkalis, are seen to disintegrate frothily like the frothy degeneration of protoplasm when attacked by alkali.

The effect of neutral salts in small concentrations on electrically charged drops of coacervate is to deprive the drops of their charge; at higher concentrations the drops become oppositely charged (Bungenberg de Jong and Dekker, 1935; Bungenberg de Jong, 1937a, b). Similar phenomena have frequently been observed in experiments on the cataphoresis of yeast cells, erythrocytes, spermatozoids, etc. (Milovanov, 1935; Rubinshtein, 1949b).

All coacervates (simple and complex) are extremely sensitive to changes in temperature. A drop in temperature as a rule causes compression of the coacervates accompanied by the appearance within them of numerous vacuoles, which subsequently merge into one large vacuole. The viscosity of the coacervate increases simultaneously. When the temperature falls below 36°, coacervates containing gelatin gelate with the formation of numerous vacuoles. Certain gelatin-containing coacervates (for example, the coacervate of gelatin and resorcin) cannot gelate. These changes in the coacervates are easily reversible on a rise in temperature (Bungenberg de Jong, 1937a, b).

Neutral non-electrolytes, especially alcohols, aldehydes and ketones, have the effect of compressing complex coacervates, making them lose part of their water. Simple coacervates react in various ways to non-electrolytes, depending on the length of the hydrocarbon chain of the non-electrolyte and on the presence of solvated groups in it. For example, methyl, ethyl and propyl alcohols cause a sodium oleate coacervate to swell, whereas n-butyl,

n-amyl, n-hexyl and n-heptyl alcohols have the opposite effect (compression). Acetone and metylethylketone cause swelling and diethylketone the opposite. In some alcohols the effect depends on the degree of branching of the chain: n-butyl alcohol causes compression of a drop, but tertiary butyl alcohol on the contrary makes it swell. The introduction of a non-electrolyte molecule may (presumably owing to the van der Waal's forces) increase the cohesive forces of the colloidal particles of a coacervate, making them cling closer to one another and expel water. This effect will be the more pronounced, the longer the hydrocarbon chain of the non-electrolyte. The compressive power of a non-electrolyte will fall in relation to the number of solvated groups—hydroxyl, oxygen, etc.—in the molecule of non-electrolyte.

The effect of non-electrolytes on simple coacervates (composed of sodium oleate and phosphatides) has been studied in detail by Bungenberg de Jong (1937c, 1938a, b, c), Bungenberg de Jong, Booij and Saubert (1937), Bungenberg de Jong and Saubert (1937) and Bungenberg de Jong, Saubert and Booij (1938).

If the contents of the cell really do form a system of coacervates, then in explaining the mechanism of the distribution of water between the cell and the medium and also of the mechanism regulating the water content of the protoplasm, we must inevitably take into account all the factors affecting the water content in the coacervates of which we have just spoken.

In the process of the biological transformation of substances within the cell and also in the process of the exchange of substances between the cell and the medium there must be a number of different substances in the protoplasm that somehow or other affect the hydration or the magnitude of the charge of the colloidal particles and hence affect their solubility. It may be supposed that it is this that forms the basis of the regulation of the water content of the protoplasm. In my opinion, it is only in this sense that one can interpret the facts presented in the preceding chapter concerning the dehydration of cells as a result of the penetration into them of substances from the surrounding medium.

*Viscosity.* The viscosity of coacervates varies within very wide limits. The relative viscosity of a complex coacervate made up of gelatin and gum arabic is between 39·6 and 46·4 at a temperature of 40°, while that of a simple coacervate of gelatin and propyl alcohol is between 525 and 526.

Many complex coacervates have an infinitely high viscosity and can be treated as solids. The viscosity of coacervates changes considerably with temperature, the effect of electrolytes and non-electrolytes and also with changes in pH. All those factors that cause coacervates to swell reduce their viscosity, whereas, on the other hand, those causing compression have the effect of raising the viscosity (Bungenberg de Jong and Dekker, 1935).

It is interesting that the viscosity of the protoplasm and many structural formations in the cell varies within the same limits as that of coacervates. As a rule, the viscosity of the protoplasm of various cells ranges within wide

limits—from a few units to many thousands. The viscosity of the protoplasm and cell nucleus depends on the physiological state of the cell. It can change tens and hundreds of times in the various periods of the development of the cell and as a result of the action of cell irritants (Heilbrunn, 1928; Nasonov and Aleksandrov, 1940; Makarov, 1948a).

*Surface tension.* Very little attention has been given to the question of the surface tension of coacervate drops. We have only fragmentary information about a few coacervates (Dobry, 1938; Ruiter and Bungenberg de Jong, 1947, Dervichian, 1949). Thus, the surface tension of a gelatin and gum arabic coacervate at its boundary with the equilibrated liquid varies from 1.46 to 2.31 dynes/cm, depending on the ratio of the amounts of the two substances at constant pH. In other conditions the same coacervate will have a different surface tension. Coacervates made of other materials have a surface tension ranging from 0.0025 to 0.02 dynes/cm and higher.

Numerous measurements have shown the surface tension of animal and plant cells to be very low. Thus, the surface tension of sea urchin eggs is 0.1–0.2 dynes/cm, that of the polychaeta worm *Chaetopterus* 0.33 dynes/cm, of amoeba 1–3 dynes/cm, of rabbit macrophages 2 dynes/cm, of leucocytes 1.3 dynes/cm, and of the protoplasm of the plasmodium *myxomycetes* 0.5 dynes/cm. It is very difficult to measure the surface tension of the protoplasm, because with the existing methods it is almost impossible to take into account the elastic properties of certain of the structural formations in the cell. In all probability, the surface tension of the protoplasm of cells is still lower than the values given in the literature (Harvey and Danielli, 1939; Höber, 1945; Makarov, 1948a).

*Colloidal films at the boundary between phases.* At the boundaries separating drops of coacervate and its equilibrating solution, the contents of vacuoles and the coacervate, or drops of one coacervate from another enclosed in the first, colloidal films are always formed, consisting of oriented colloidal particles of that colloid which is present in excess in the system coacervate/equilibrating surrounding liquid. These colloidal films determine the charge on the coacervate drop, the vacuoles, and solid particles or drops of organic liquid that pass into the coacervate. These colloidal envelopes are not stable and have the property of admitting through themselves dissolved crystalloids, colloidal particles and coarse suspensions.

The envelopes that separate the nucleus from the protoplasm, the nucleolus from the karyoplasm, are evidently of the same nature. They occur, obviously, on chondriosomes, karyosomes, chromosomes and other organoids and inclusions in cells.

*The distribution of the components of a coacervate in the system coacervate/equilibrating solution.* The contents of a coacervate are in dynamic equilibrium with the surrounding liquid. The colloidal substances and mineral ions that go to make up the coacervate complex can, under certain conditions (change of pH, salt concentration, temperature of the equilibrated liquid), pass into

the surrounding solution or into vacuoles that form as a result of the new conditions, or, on the other hand, they may be absorbed by the coacervate. This phenomenon is determined by the degree of hydration and the magnitude of the charges of the colloidal particles. If the surrounding liquid is acidified, colloidal particles of gelatin, albumin and other proteins which form part of the coacervate complex acquire increased positive charge, and consequently colloidal particles of a negatively charged colloid are absorbed from the surrounding liquid. If, however, the liquid is strongly acidified, its positive charge may be drastically reduced, which will cause the emission of protein from a coacervate and even its decomposition. Other strong electrolytes have an analogous effect on the composition of coacervates. The question has been studied in detail by Bungenberg de Jong and Dekker (1935, 1936) and Bungenberg de Jong (1947).

At certain stages in the development of a cell or under the action of various agents substances which are vitally important components of the cell are often seen to undergo a change of state in the protoplasm: lipids, nucleic acids, enzymes, etc. As a rule all these substances have labile bonds with the fundamental substrate of the protoplasm—protein. It may be supposed that they form part of the protoplasm complex and can undergo change in certain physiological states of the latter.

Coacervates have one more important property: they can accumulate certain substances out of dilute solutions in great quantities and, on the other hand, admit only limited amounts of other substances dissolved in the surrounding liquid. This property results from the fact that the colloidal particles of a coacervate can absorb solutes and that the solubility of these substances in the water of the coacervate is considerably reduced because the water is in the "bound state" (Oparin, 1957). Such a mechanism of the distribution of substances between a coacervate and its surrounding liquid is of great significance for the theory of permeability and will be discussed in detail in the following chapter.

Coacervate drops can absorb solid particles (Indian ink, collargol), drops of various organic liquids which are immiscible with water, bacteria, erythrocytes and various other particles and hold them as inclusions. The whole process of the absorption of these particles bears a striking external resemblance to the analogous phenomenon in the world of single-cell organisms, leucocytes, etc. (Bungenberg de Jong and Kruyt, 1930; Bungenberg de Jong, 1932, etc.).

#### 4. *Conclusions*

A number of the features which characterise the physico-chemical properties of coacervates are also characteristic for the protoplasm. The viewpoint of many workers, that the protoplasm of living cells is a system of complex coacervates, has therefore a firm experimental basis.

In the physiology and the morphology of cells there is continually more work

being published in which the writers try to explain some observed cellular phenomenon in terms of the transformation of coacervates and change in their properties as a result of some external factor or because of some internal forces associated with the mitosis of the cell. However, the number of such published works is still extremely small. The reason for this is, evidently, to be found in the fact that the prevailing conception in physiology has, up to the present, been that of the protoplasm as an aqueous solution of colloidal substances with the structure of a sol or gel. This concept inevitably drives the research worker to conclude that there is on the surface of the cell a membrane which is fundamentally different in its physico-chemical properties from the inner layers of the protoplasm and causes them to explain many biological phenomena in terms of these membranes and their properties, making the protoplasm itself take second place in these phenomena.

## CHAPTER IV

### The Distribution of Substances between a Coacervate and its Surrounding (Equilibrated) Fluid

IN THE last chapter we reviewed the facts that point to the protoplasm being a system of coacervates. If this is in fact so, it is important to explain the causes governing the absorption by a coacervate of substances dissolved in its surrounding liquid so as to understand more deeply the laws that lie at the basis of the phenomena of cell permeability. Bungenberg de Jong (1932), followed by Oparin (1945) and Nasonov (1949a), has pointed out the importance of studying the distribution of substances between coacervates and their media in the investigation of the permeability of cells.

TABLE 9. COMPOSITION OF THE COACERVATE SYSTEM; GELATIN + SODIUM SULPHATE + WATER IN PER CENT. (from Holleman et al., 1934)

	Medium			Coacervate			Temperature (°C)
	Gelatin	Water	Sod. Sulph.	Gelatin	Water	Sod. Sulph.	
1	2·8	87·2	9·4	10·4	82·1	7·5	50·04
2	0·7	87·1	12·2	24·1	69·7	6·2	50·04
3	0·2	85·0	14·8	27·2	66·6	7·2	50·04
4	0·2	82·4	17·4	36·7	57·0	6·3	50·04
5	0·8	87·0	12·2	26·1	67·6	6·3	60·02

Holleman, Bungenberg de Jong and Modderman (1934) studied the distribution of alcohol, resorcin, sodium sulphate and potassium iodate between a simple coacervate of gelatin and its surrounding liquid. They established that if a substance is adsorbed by the colloidal particles of gelatin (for example, like resorcin and potassium iodate), then there will be a larger concentration of it in the coacervate than in the surrounding liquid, whereas, if it is not adsorbed, there will be less in the coacervate than in the surrounding medium (for example, sodium sulphate and, in certain concentrations, alcohol). By way of illustration, some of the analytical results obtained by these workers are reproduced in Tables 9 and 10. The data in these tables were used by myself to calculate the concentrations of resorcin and sodium sulphate as given in Table 11. These were calculated in grams per 100 g of

coacervate water ( $C_c$ ) and per 100 g water of the surrounding liquid ( $C_s$ ). The ratio of these quantities ( $Q$ ) is also given in this table.

Comparison of the  $Q$  values given in the table shows that the concentration of sodium sulphate in the surrounding liquid is greater than in the coacervate, and that with increasing concentration of this electrolyte in the medium this difference grows continuously: from 0·82 at  $C_s = 10\cdot8$  per cent  $Q$  falls to 0·52 at  $C_s = 21\cdot2$  per cent, that is, in the latter case there is 48 per cent

TABLE 10. COMPOSITION OF THE COACERVATE SYSTEM: GELATIN + RESORCINOL IN PER CENT  
(from Hollemann *et al.*, 1934)

	Medium*		Coacervate			Temperature (°C)
	Water	Resorcinol	Gelatin	Water	Resorcinol	
1	95·8	4·2	25·7	66·4	7·9	45
2	94·1	5·9	30·3	58·1	11·6	45
3	82·5	17·5	27·0	48·4	24·6	45
4	69·6	30·4	13·3	51·8	34·9	45

\* Gelatin content—traces.

TABLE 11. THE DISTRIBUTION OF SODIUM SULPHATE AND RESORCINOL BETWEEN THE COACERVATE AND ITS MEDIUM (from the data of Tables 9 and 10)

	Sodium Sulphate		$Q = \frac{C_c}{C_s}$	Resorcinol		$Q = \frac{C_c}{C_s}$
	% of content in the water of the medium $C_s$	% of content in the water of the coacervate $C_c$		% in the medium $C_s$	% in the coacerv- ate $C_c$	
1	10·8	9·1	0·83	4·2	10·7	2·55
2	14·0	8·9	0·64	5·9	14·5	2·50
3	17·4	10·7	0·61	17·5	33·7	1·90
4	21·2	11·1	0·52	30·4	40·1	1·33

less sodium sulphate in the coacervate than in the balanced liquid. The difference in the distribution in the case of resorcin is striking. Here the concentration in the coacervate is considerably greater than in the surrounding liquid.  $Q$  in this case is greater than unity. However, as the concentration of resorcin in the surrounding liquid increases,  $Q$  falls just as in the case of sodium sulphate (from 2·55 at  $C_s = 4·2$  per cent to 1·33 at  $C_s = 30·4$  per cent).

Bungenberg de Jong and his colleagues find two causes to explain this phenomenon. First, the coacervate contains only "bound" (solvated) water, for which reason its activity as a solvent is reduced. This causes the observed

preponderance of solute in the surrounding liquid. Second, substances that are adsorbed by the colloids of the coacervate can be accumulated in it, and this can lead to a considerable preponderance of these substances in the coacervate.

The fall in the value of  $Q$  for sodium sulphate with increasing concentration is, evidently, to be explained by the fact that there is a simultaneous sharp reduction in the water content of the coacervate. Because the first molecules to emerge from the coacervate will be the water molecules that are less firmly bound by the colloids, this must result in a reduction of the dissolving power of the remaining water.

In the example of resorcin, it can clearly be seen that the preponderance of this substance in the coacervate is caused by its adsorption by the colloids. In view of the fact that the adsorption of a substance by an adsorbent does not increase proportionally to its increase in the external liquid (because there is a limit to adsorption), we obtain a reduction in  $Q$  for increasing  $C_s$ .

Bungenberg de Jong and his colleagues conducted a large number of experiments to clarify the distribution of various substances between the coacervate and its surrounding liquid, obtaining results analogous to those described above.

TABLE 12. THE CHANGE IN THE CONCENTRATIONS OF GALACTOSE IN THE COACERVATE AND THE EQUILIBRATED LIQUID WITH TIME. TEMPERATURE 40°.

Length of Experiment (hr)	Concentration of Galactose (in g per 100 ml of water)		$Q = \frac{C_c}{C_s}$
	in the coacervate ( $C_c$ )	in the medium ( $C_s$ )	
15	0·39	0·33	
15	0·43	0·38	
15	0·38	0·37	
Mean	0·40	0·36	1·11
18	0·41	0·31	
18	0·36	0·38	
18	0·37	0·36	
18	0·38	0·31	
Mean	0·38	0·34	1·12
15	2·13	2·76	
15	2·07	2·84	
Mean	2·10	2·80	0·75
18	2·07	2·89	
18	1·98	2·96	
18	2·01	2·94	
Mean	2·02	2·93	0·69

Thus, judging by the data obtained by Bungenberg de Jong and his colleagues, we find that two sorptional factors—solubility and adsorptional binding—determine the whole complex picture of the distribution of substances between a coacervate and its surrounding liquid. However, the results of these experiments do not yet allow us to conclude to what extent the distribution of a substance in the system: coacervate/surrounding liquid depends on solubility and adsorption, because the substance whose distribution was studied was also the agent causing the coacervation of the colloid, and, with increasing concentration of this agent, the water content of the coacervate was changed together with the solubility of the agent in it.

To explain the role of solubility and adsorption in the distribution of substances between a coacervate and its medium, I have studied the distribution of galactose and sucrose between a complex coacervate and its balanced liquid (Troshin, 1951a). As was shown by Bungenberg de Jong and Dekker (1929), sugars in small concentrations do not have any substantial effect on the process of coacervation or on the composition and properties of the coacervate.

The complex coacervate chosen for these experiments was that made from gelatin and gum arabic.

The experimental procedure was as follows. 40 ml of a 2 per cent gelatin solution were poured into a dropping funnel together with a like quantity of gum arabic solution and 10 ml of sugar solution. The whole was mixed, and heated to 35°; then a further 10 ml of 0.1 N hydrochloric acid were added, after which the mixture was put in a thermostat at 40°.

Next day the coacervate was separated from the surrounding liquid. Then in both fractions (the coacervate and the surrounding liquid) I determined the amounts of water, dry residue (by desiccation to constant weight) and the sugar concentrations.

First the time was determined at which diffusion equilibrium was reached between the sugar dissolved in the surrounding liquid and that in the coacervate. From the data in Tables 12 and 13 it follows that the diffusion equilibrium of galactose and sucrose in the system coacervate/surrounding liquid is achieved in less than 15 hr. All subsequent experiments with coacervate systems were therefore continued for 17–19 hr. A shorter term could have been chosen; but in this case it would not have been certain that there would be complete precipitation of the smallest coacervate droplets out of the surrounding fluid onto the bottom of the funnel.

The figures in Tables 12 and 13 show that in conditions of diffusion equilibrium the ratio of the galactose and sucrose concentrations in the coacervate to their concentrations in the surrounding liquid changes markedly depending on the sugar concentration in the surrounding liquid.

This dependence can be strikingly expressed by the ratio of the sugar concentration in the coacervate ( $C_c$ ) to the sugar concentration in the surrounding liquid ( $C_s$ ):  $Q = C_c/C_s$ . When the galactose concentration in the

surrounding liquid is 0.34–0.36 per cent,  $Q > 1$ , while if the concentration is 2.80–2.93 per cent,  $Q \leq 1$ , while for sucrose, if  $C_s$  is between 0.19 and 0.21 per cent,  $Q > 1$ , whereas if  $C_s$  is between 3.07 and 3.14 per cent,  $Q < 1$ .

TABLE 13. THE CHANGE IN THE CONCENTRATIONS OF GALACTOSE IN THE COACERVATE AND THE EQUILIBRATED LIQUID WITH TIME. TEMPERATURE 40°.

Length of Experiment (hr)	Concentration of Galactose (in g per 100ml of water)		$Q = \frac{C_c}{C_s}$
	in the coacervate ( $C_c$ )	in the medium ( $C_s$ )	
15	0.21	0.21	
15	0.20	0.18	
15	0.23	0.17	
15	0.20	0.19	
15	0.21	0.20	
Mean	0.22	0.19	1.16
18	0.28	0.21	
18	0.25	0.21	
18	0.26	0.20	
18	0.25	0.22	
Mean	0.26	0.21	1.24
15	2.15	3.17	
15	2.11	3.11	
Mean	2.13	3.14	0.68
18	2.14	3.08	
18	2.06	3.10	
18	2.05	3.15	
18	2.08	3.07	
Mean	2.08	3.07	0.67

To obtain a detailed explanation of this dependence another series of experiments was performed with intermediate sugar concentrations. The experiments again lasted 17–19 hr.

The data obtained from these experiments are given in Table 14. They indicate that within wide limits of variation of the sugar concentrations in the balanced liquid, the amount of dry residue and water in the coacervate and the balanced liquid varies very little. Hence it follows that galactose and sucrose in the concentrations used have no essential effect on the hydration of the colloidal particles of the coacervate and the degree of completeness with which the micelles of gum arabic and gelatin enter into the process of the formation of their complexes. Only with a sucrose concentration in the coacervate of about 10 per cent is the amount of colloids in it about

halved, while a further increase in the concentration causes the coacervate to disintegrate or not to form at all. This circumstance has a vital effect on the distribution of sucrose between the coacervate and the surrounding liquid, as can clearly be seen from the ratio of the concentration of this non-electrolyte in the coacervate to its concentration in the surrounding liquid. Table 14 shows the concentration of galactose and sucrose in per cent in relation to the water in the coacervate and the surrounding liquid. This table also incorporates the data in Tables 12 and 13, which were obtained in experiments lasting 18 hr. Comparing the sugar concentration in the coacervate ( $C_c$ ) with its concentration in the surrounding liquid ( $C_s$ ), we see that with increasing  $C_s$ ,  $C_c$  also increases, but not proportionally to the increase in the concentration of non-electrolyte in the surrounding liquid. It is further found that the sugar concentration in the coacervate can be either greater or

TABLE 14. THE DISTRIBUTION OF GALACTOSE AND SUCROSE BETWEEN THE COACERVATE AND MEDIUM. TEMPERATURE 40–41°. THE MEAN VALUE OF 4–10 EXPERIMENTS IS GIVEN

	Composition of the coacervate			Composition of the medium liquid			$Q = \frac{C_c}{C_s}$
	Water (%)	Dry residue (%)	Sugar (g per 100ml water $C_c$ )	Water (%)	Dry residue (%)	Sugar (g per 100ml water $C_c$ )	
Galactose							
1	84.6	15.4	0.25	99.2	0.8	0.19	1.32
2	84.5	15.5	0.38	91.1	0.9	0.34	1.12
3	84.1	15.9	0.66	98.7	1.3	0.74	0.87
4	83.9	16.1	1.12	98.1	1.9	1.47	0.76
5	82.3	17.7	2.02	96.5	3.5	2.93	0.69
Sucrose							
1	85.1	14.9	0.26	99.1	0.9	0.21	1.24
2	84.9	15.1	0.57	98.7	1.3	0.58	0.98
3	84.3	15.7	0.87	98.3	1.7	1.14	0.77
4	84.0	16.0	1.42	98.1	1.9	2.02	0.70
5	83.7	16.3	2.08	96.3	3.07	3.07	0.67
6	83.8	16.2	3.76	94.1	5.9	4.75	0.79

less than in the medium, depending on the sugar content of the latter phase. Thus, for galactose, when  $C_s = 0.19$  per cent,  $Q = 1.32$ , i.e., there is 32 per cent more galactose in the coacervate than in the balanced liquid, while when  $C_s = 2.93$  per cent,  $Q = 0.69$ . In other words, in this case the concentration of galactose in the coacervate is less by 31 per cent. Analogous results were obtained in the experiments with sucrose, as can be seen from Table 14.

The dependence of  $Q$  on the concentration of sugars in the balanced liquid is shown in Figs. 29 and 30.

It can be seen from these figures that  $Q$  decreases with increasing  $C_s$ . The dotted lines, corresponding to equal sugar concentrations in the coacervate and the medium ( $Q = 1$ ), differ greatly from the experimental curves. They show that when the galactose concentration in the medium is greater than 0·41 per cent,  $C_c < C_s$ , while if the concentration is less than 0·41 per cent,  $C_c > C_s$ .

Figure 30, which shows the dependence of  $Q$  on the sucrose concentration in the medium ( $C_s$ ), reveals that when  $C_s$  is less than 0·5 per cent, there is

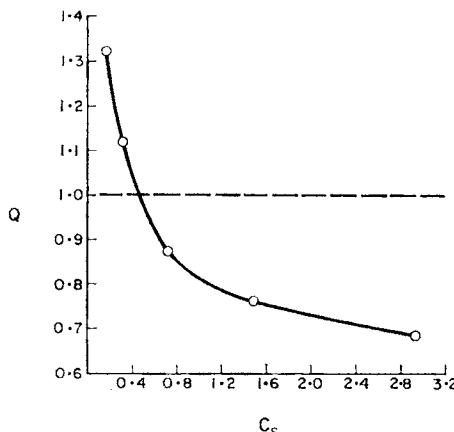


FIG. 29. The dependence of the coefficient  $Q$  on the galactose concentration in the medium ( $C_s$  in per cent) when galactose is distributed between the coacervate and the equilibrated liquid.

more non-electrolyte in the coacervate than in the medium ( $Q > 1$ ). As  $C_s$  increases to 3 per cent,  $Q$  rapidly falls, becomes less than unity and only when  $C_s = 4\cdot75$  does it again rise, which is explained, apparently, by the change of the coacervate itself.

Such a relation between the sugar concentration in the coacervate and in the medium is evidently to be explained by the fact that the presence of these non-electrolytes in the coacervate is determined by their ability to be adsorbed by the colloids and by their lowered solubility in the water of the coacervate, as happens in the case of sodium sulphate and resorcin. The conjunction of these factors—adsorption and lowered solubility—determines the various magnitudes of the ratio  $Q$ . If this is so, then the total amount of sugar in the coacervate ( $C_c$ ) is composed of the sugar dissolved in the water of the coacervate ( $C$ ) and the adsorbed sugar ( $A$ ), or

$$C_c = C + A. \quad (1)$$

If the amount of water in the coacervate remains substantially unchanged with a change in the concentration of the non-electrolytes, then it may be

assumed that the properties of its aqueous phase as a solvent also do not change, at least within the limits of the variation of the sugar content of the balanced liquid that occurred in my experiments.

In these conditions the relation between the concentration of dissolved sugar ( $C$ ) in the water of the coacervate to its concentration in the balanced liquid ( $C_s$ ) can be expressed by the following formula:

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

where  $K$  is the coefficient of proportionality characterising the properties of the aqueous phase of the coacervate as a solvent. The coefficient  $K$  can be

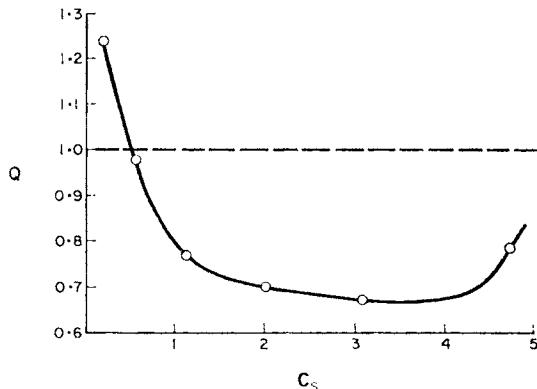


FIG. 30. The dependence of the coefficient  $Q$  on the concentration of sucrose in the medium ( $C_s$ , in per cent) when sucrose is distributed between the coacervate and the medium.

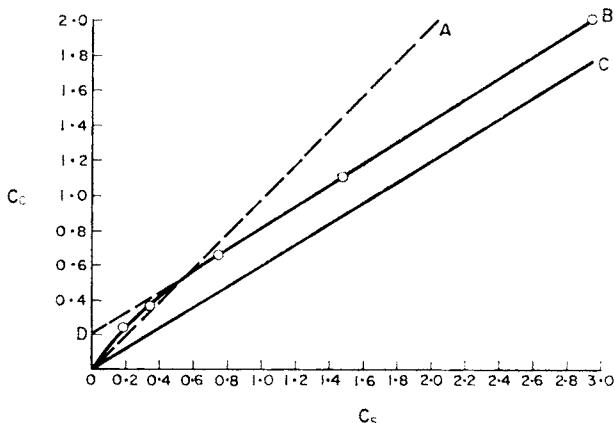


FIG. 31. The dependence of the galactose concentration in the coacervate ( $C_c$ , in g per 100 ml of coacervate water) on its concentration in the medium ( $C_s$ , in per cent).

treated as the distribution coefficient of Henry's equation, which reflects the laws of the distribution of substances between two phases. Substituting in (1) for  $C$  from (2), we obtain:

$$C_c = C_s K + A. \quad (3)$$

In this equation  $A$  (the adsorbed sugar) is not a constant, but varies depending on the concentration of the sugar dissolved in the water of the coacervate ( $C$ ), which, according to (2), is equal to  $C_s K$ . The dependence of  $A$  on  $C_s K$  can be expressed by Langmuir's equation for non-polar adsorption:

$$A = A_\infty \frac{C_s K}{C_s K + a}, \quad (4)$$

where  $A_\infty$  is the limit of adsorption and  $a$  a constant characterising the curvature of the rise in the adsorption isotherm.

Substituting now in (3) the value of  $A$  from (4), we obtain:

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

For a sufficiently large concentration of sugar in the medium, when  $a$  becomes small compared with  $C_s K$ , the ratio  $C_s K / C_s K + a$  approaches unity, and then equation (5) may be written in the form:

$$C_c = C_s K + A_\infty. \quad (6)$$

The data obtained by us (Table 14) permit us to find all the coefficients in equation (5) and to determine with sufficient accuracy the amount of non-electrolytes in the coacervate in the dissolved state and in the adsorbed. This is easily done graphically.

In Figs. 31 and 32 the curves OB show the distribution of galactose and sucrose between the coacervate and the medium as given by the data of Table 14. The sugar concentration in the medium ( $C_s$ ) is plotted along the abscissae, while its concentration in the coacervate in relation to the water in it ( $C_c$ ) is plotted along the ordinate. If the solubility of the sugars in the water of the coacervate were the same as in the water of the balanced liquid, and if the sugar were not adsorbed by the colloids of the coacervate, then we should have a relation corresponding to the bisector OA. In fact the distribution curve OB runs at first above this line ( $C_c > C_s$ ), then intersects it, becomes a straight line and runs under the line at a definite angle to the abscissae ( $C_c < C_s$ ). The straight line DB, whose left part is a continuation of the straight portion of OB, corresponds to equation (6). The intercept on the ordinate OD is numerically equal to the adsorption limit  $A_\infty$  in equations (5) and (6). In this case it equals 0.22 g per 100 ml of coacervate water,

which corresponds to a content of 18.0–19.3 g dry residue of gelatin and gum arabic (see Table 14).

The straight line OC, parallel to DB, will reflect the dependence of the amount of dissolved sugar in the coacervate ( $C_c - A$ ) on its concentration in the medium, which corresponds to Henry's law—equation (2). Using the graph to determine the value of  $C$  corresponding to any value of  $C_s$ , we find from equation (2) the magnitude of the distribution coefficient  $K$ . In our case,  $K = 0.61$  for galactose and  $K = 0.60$  for sucrose. In other words,

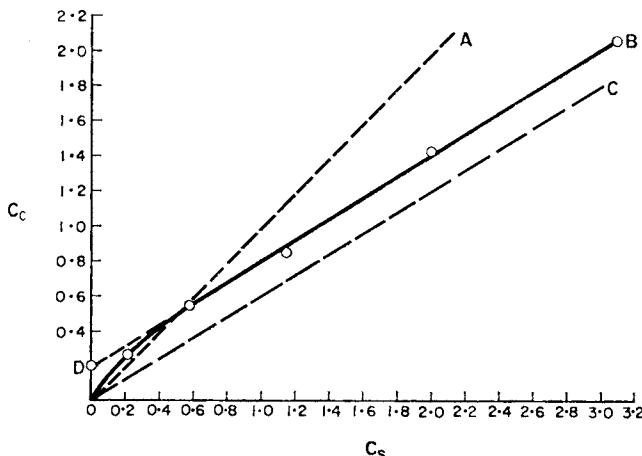


FIG. 32. The dependence of the sucrose concentration in the coacervate ( $C_c$ , in g per 100 ml of coacervate water) on its concentration in the medium ( $C_s$ , in per cent).

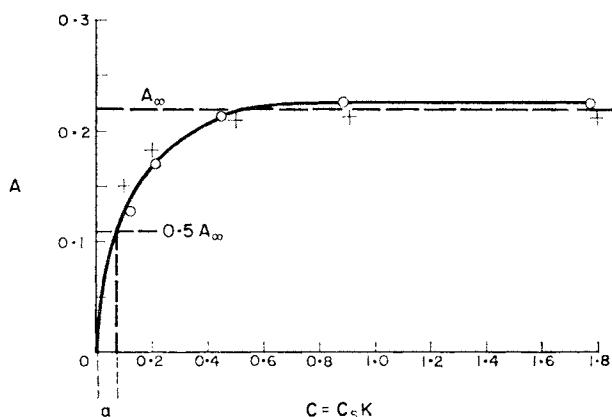


FIG. 33. The isotherm (of the adsorption) of galactose by the coacervate colloids. Along the abscissae the concentrations of galactose dissolved in the coacervate water ( $C = C_s K$ , in per cent), ordinates the amount of adsorbed galactose in the coacervate ( $A$ , in g).

the solubility of galactose in the water of the coacervate is 39 per cent, and that of sucrose 40 per cent, less than in the water of the medium.

Knowing the value of  $K$ , we can determine from (2) the concentration of the dissolved sugar in the coacervate ( $C$ ) from a given value of  $C_s$ . This allows us to find also the amount of adsorbed sugar in the coacervate, for which it suffices to subtract the dissolved sugar from the total sugar ( $C_c$ ) i.e. ( $A = C_c - C_s K$ ).

TABLE 15. THE QUANTITY OF DISSOLVED AND ADSORBED SUGAR IN THE COACERVATE IN RELATION TO ITS CONCENTRATION IN THE MEDIUM (in per cent)

	Concentration of sugar		Of the total sugar in the coacervate	
	equilibrating in the liquid ( $C_s$ )	in the coacervate ( $C_c$ )	amount dissolved ( $C = C_s K$ )	amount adsorbed ( $A = C_c - C$ )
Galactose				
1	0·19	0·25	0·12	0·13
2	0·34	0·38	0·21	0·17
3	0·74	0·66	0·45	0·21
4	1·47	1·12	0·89	0·23
5	2·93	2·02	1·79	0·23
Sucrose				
1	0·21	0·26	0·13	0·13
2	0·58	0·57	0·35	0·22
3	1·14	0·87	0·68	0·19
4	2·02	1·42	1·21	0·21
5	3·07	2·08	1·84	0·24

Table 15 shows how much of the sugar in the coacervate is adsorbed and how much dissolved.

Figures 33 and 34 depict respectively the adsorption isotherms of galactose and sucrose, drawn from the data of Table 15. The concentration of dissolved sugar ( $C$ ) is plotted along the abscissae, and the amount of adsorbed sugar in the coacervate along the ordinate (in g per 100 ml of water or per 18·0 to 19·3 g dry residue of the coacervate). It is not difficult to find the constant  $a$  in equation (5) from the isotherm curve. As is well known, it is equal to that concentration of the adsorbed substance in the external liquid ( $C$ ), for which  $A = 0·5 A_\infty$ . In the present case  $a = 0·04 C_s K$  for galactose and sucrose.

On the basis of the data obtained I came to the conclusion that the concentration of sugars in the coacervate in relation to their concentration in the medium is determined by the lowered solubility of these substances in the water of the coacervate (in comparison with their solubility in the water of

the medium) and by the adsorption of these non-electrolytes on the surface of the colloidal particles. At small concentrations of sugar in the medium the dominant factor is adsorption, which can cause the concentration of non-electrolyte in the coacervate to exceed its concentration in the medium, while

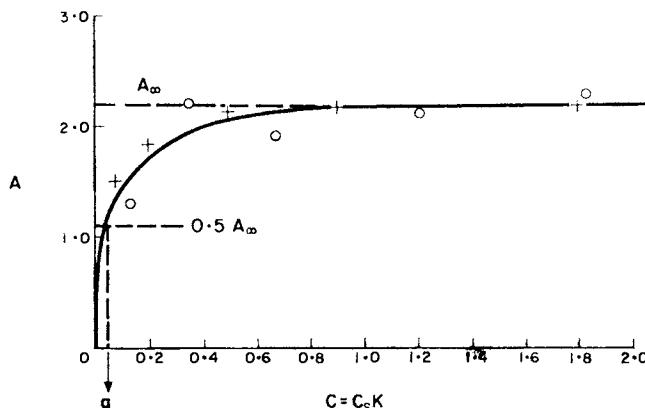


FIG. 34. The isotherm of the adsorption of sucrose by the coacervate colloids.

at large concentrations first place is taken by the reduced solubility of the substance in the water of the coacervate, as a result of which there can be less sugar in it than in the medium.

We shall see further that this law of the distribution of substances is completely applicable to live cells as well.

## CHAPTER V

# The Permeability of Cells for Non-Electrolytes

### A. ANIMAL CELLS

#### 1. *The Permeability of Cells for Sugars*

*Erythrocytes.* Many workers who have studied the permeability of cells for sugars by means of the osmotic methods have come to the conclusion that pentoses, hexoses, disaccharides and polysaccharides are completely unable to penetrate the erythrocytes of the majority of animals (Grijns, 1896; Hedin, 1897, 1898; Masing, 1913, 1914a, b; Kozawa, 1913, 1914; Ege, 1920a, b, 1921a, b; Mond and Hoffmann, 1928b; Fleischmann, 1928; Mond, 1930, and others).

On the other hand, a number of writers using chemical methods for these investigations have reached the opposite conclusion.

Thus, Rona and Michaelis (1909) and Rona and Döblin (1911) found by chemical analysis that glucose added to human blood is distributed in *in vitro* experiments between the formed elements and the plasma. Since that time a large amount of work has been done with chemical techniques on the permeability of the erythrocytes of various animals.

In the study of the permeability of cells by this method a criterion frequently used has been the coefficient of the distribution of the substance between the cell and the medium ( $Q$ ):\*

$$Q = \frac{\text{the concentration of the substance in the cell}}{\text{the concentration of the substance in the medium}} .$$

If  $Q$  for one substance reaches over a definite time interval a greater value than for another in the same interval, then we can say that the permeability of the cell for the first substance is greater than for the second. If the distribution coefficient  $Q$  reaches unity in a finite period, this is often spoken of as "complete permeability" of the cells.

\* In speaking of the distribution of substances between cells and the medium we have in mind the processes of solution, adsorption and chemical interaction which cause one or other concentration of the substance in the cell. This concept must not be confused with the distribution of a substance between two phases, which is based on the solubility of the substance in both phases only. The latter process obeys Henry's law, the former does not.

Of older publications we should first consider the experiments of Masing (1913, 1914a, b). Using chemical techniques under the most varied conditions, he failed to find any penetration of grape sugar into nucleated erythrocytes of birds (geese), rabbits, pigs and sheep. Bovine and canine erythrocytes are permeable for this sugar only to a small extent, while this sugar penetrates human erythrocytes very quickly: diffusion equilibrium in 0·2–0·8 per cent solutions of grape sugar is established in 15–20 min,  $Q$  in this time attaining a value of 0·6–0·7 ( $Q$  falls with increasing sugar concentration in the medium, see Table 16).

TABLE 16. THE DISTRIBUTION OF DEXTROSE BETWEEN HUMAN ERYTHROCYTES AND THEIR EQUILIBRATED LIQUID (from Masing, 1914a).

Expt. No.	Length of experiment	Sugar concentration (g per 100 ml)		$Q = \frac{C_c}{C_s}$
		in the medium ( $C_s$ )	in the erythro- cytes ( $C_c$ )	
1	14 hr	0·20	0·14	0·70
2	35 min	0·68	0·44	0·65*
3	55 min	0·80	0·50	0·63*
4	2 hr	0·90	0·60	0·67*
5	75 min	1·00	0·61	0·61
6	1 hr	2·80	1·46	0·52*
7	50 min	2·96	1·57	0·53
8	4 hr	2·98	1·64	0·55

\* The sugar concentration in the erythrocytes was calculated from the loss of the sugar in the medium.

According to Masing, pentoses and hexoses rapidly penetrate human erythrocytes, but disaccharides (lactose, sucrose and other sugars) are unable to penetrate, a finding that is, however, contradicted by other workers.

On the basis of osmotic experiments and chemical analysis, Kozawa (1914) concluded that human erythrocytes and those of the monkey, dog, cat, rabbit, ox, pig, goat, sheep, horse and guinea pig are impermeable for disaccharides, while monosaccharides (fructose, levulose, glucose, sorbose, galactose, mannose, xylose and arabinose) penetrate only human, monkey and canine erythrocytes. However, the analytical data given by the author in this work show that even disaccharides (sucrose, maltose and lactose) are absorbed in small quantities by human, dog and pig erythrocytes, while pentoses and hexoses penetrate not only human, monkey and canine erythrocytes but also those of cats, pigs and rabbits. Ege (1920a, 1921a, b) observed that glucose when added to rabbit blood does not penetrate the erythrocytes, whereas it quickly penetrates canine and human erythrocytes; he established that the distribution coefficient,  $Q$ , is 0·33 for canine erythro-

cytes and 0·75 for human. Ege also discovered that the absorption of glucose by human erythrocytes finishes before they begin to swell in its isotonic solution, from which he concluded that the sugar is first adsorbed on the surface of the erythrocyte and then slowly penetrates within the cell. Animal blood cells of other types are not, in general, penetrated by this sugar, which is only adsorbed on their surface.

In view of this some writers consider that the chemical method in general is not suitable for the study of the permeability of cells (Fleischmann, 1928; Mond and Hoffmann, 1928b; Mond, 1930; Gellhorn, 1929; Rubinshtain, 1947, and others). However, the observed rise in glycolysis on the absorption of sugar by erythrocytes clearly indicates that the sugar penetrates

TABLE 17. THE NORMAL SUGAR CONTENT OF HUMAN AND ANIMAL BLOOD  
(in mg per cent, mean of 4–7 analyses). After Shope (1928a, b).

Subject	Sugar in the erythrocytes	Sugar in the plasma	Sugar in whole blood	% erythrocytes of volume of whole blood	% sugar in erythrocytes of total blood sugar content
Pig	38	109	76	48	24
Guinea pig	139	137	138	48	48
Ox	30	55	45	43	30
Rabbit	37	115	81	43	20
Human	121	103	111	45	49

the erythrocyte, because there is no ground for supposing, as has rightly been pointed out by Kolotilova and Engel'gardt (1937), Brooks (1947) and others, that such a complex enzymatic process takes place on the surface of cells.

Folin and Berglund (1922a) and Wu (1922) found that fermenting sugars are normally distributed in human blood in equal proportions between the plasma and the formed elements. Thus, according to Wu, 99·9 mg per cent sugar was found in human erythrocytes and 103·2 mg per cent in the plasma, these figures being the average of a number of analyses. According to Folin and Berglund, there is 94–95 mg per cent sugar in the blood of a man fasting, 96–107 mg per cent in the plasma and 90–102 mg per cent in the erythrocytes. On the introduction of large quantities of sugar into the digestive tract the sugar content both of the blood as a whole and of the plasma and erythrocytes is increased, dropping slowly to normal thereafter. This is convincing evidence that human erythrocytes are permeable to sugar *in vivo* as well.

Further, Shope (1928a, b), using various chemical methods, determined the normal sugar content in the blood, plasma and erythrocytes of various types of animals and humans as well (Table 17).

The above mentioned data on the permeability of various animal erythrocytes for sugars contradict one another most strikingly. These contradictions evidently arise from the methodological inadequacies of the experiments. It should be pointed out that in many cases no account was taken of the speed of glycolysis, which varies in the erythrocytes of different animals, and further, no allowance was made for the fact that all methods of determining sugar concentration which are based on their reducing power give elevated results because of the reduction of non-carbohydrate substances (so-called "residual reduction").

Somogyi (1928, 1930a, b, 1931) developed an exact method for determining blood sugars in which allowance can be made for residual reduction of the plasma and formed elements. According to his data (1933), the residual reduction of erythrocytes ranges from 30 (ox) to 51 (guinea pig) mg per cent glucose, while the figure for the serum of many animals is about 7 mg per cent glucose. Ege and Roche (1930) found that the residual reduction per 100 mg human erythrocytes amounts to 25 mg per cent glucose and 10 to 12 mg per cent per 100 mg serum. 5 per cent of this reduction is at the expense of creatine, 25 per cent at the expense of creatinine, 50 per cent at the expense of uric acid and the remainder at the expense of the other non-carbohydrate substances.

Table 18 shows the normal distribution of "true" sugar (total reduction minus residual reduction) between the erythrocytes and plasma of various animals.

Ege and Roche (1930), on the basis of their own and many other published results, came to the conclusion that the ratio of the sugar concentrations in human erythrocytes and plasma is 80:100. Hence they assert that the sugar concentrations in the aqueous phases of the elements of the blood and the plasma are equal. The same conclusion was reached by Calvin (1932), MacKay (1932) and others. However, the results of Folin and Svedberg (1930) contradict this assertion. Folin and Svedberg found that the concentration of free sugar in the water of human erythrocytes is approximately half as much as in the plasma.

Svedberg studied the distribution of fermentable sugar between the formed elements and the blood plasma in 17 types of animals and obtained the following values of the coefficient  $Q$  (the calculation is per 100 ml of erythrocytes and 100 ml of plasma, about 100 mg per cent sugar being found in the plasma of all types of animals): bass -0·41, pollack -0·12, sea devil -0·88, sea dog -0·57, pigeon -0·14, rabbit -0·15, guinea pig -0·23, nutria -0·23, dog -0·21, sheep -0·11, ox -0·26, horse -0·27, dolphin -0·55, monkey -0·54. In the erythrocytes of the pig and tortoise no sugar was found.

In the opinion of this worker, the level of the sugar content in the erythrocytes of those animals where the distribution coefficient  $Q$  is greater than 0·5 is regulated by the speed of diffusion of carbohydrate into the ery-

TABLE 18. THE NORMAL DISTRIBUTION OF FERMENTABLE SUGAR BETWEEN THE ERYTHROCYTES AND SERUM IN HUMANS AND SOME TYPES OF ANIMALS (from Somogyi, 1933)

Subject	Sugar content (mg%)		$Q$
	erythro- cytes	serum	
Ox	15	85	0.18
Calf	61	136	0.45
	36	101	0.36
Sheep	13	80	0.16
	7	80	0.90
Dog	35	150	0.23
	48	115	0.42
Cat	73	263	0.28
	79	331	0.24
Rabbit	42	144	0.29
	40	147	0.27
Guinea pig	40	127	0.33
	67	187	0.36
Monkey	124	149	0.82
	122	144	0.87
	114	151	0.76
Human	80	100	0.80*

\* Mean of numerous determinations (Somogyi, 1928).

throcytes and the speed of glycolysis. Where  $Q$  is less than 0.5, and also does not increase on the exclusion of glycolysis, the sugar does not penetrate the cells but is merely adsorbed onto their surface.

The work of Engel'gardt and Kolotilova (1936), Kolotilova (1937) and Kolotilova and Engel'gardt (1937) showed that glucose penetrates human, rabbit and cat erythrocytes when washed in Ringer's solution and does not penetrate, or hardly penetrates, pig blood cells. However, Morgulis (1937)

TABLE 18b. THE DISTRIBUTION BETWEEN THE PLASMA AND THE ERYTHROCYTES OF SUGARS ON THEIR INTRODUCTION INTO THE BLOOD OF RATS (from Helmreich and Cori, 1957).

Sugar introduced	Time after introduction (min)	Sugar concentration		Volume of erythrocytes in % of whole blood	Sugar concentration in the erythrocytes (mg%) $C_c$	$Q = \frac{C_c}{C_s}$
		plasma (mg%) $C_s$	whole blood (mg%)			
d-Xylose	40	249	196	41.7	57.5	0.20
d-Arabinose	120	222	166	41.7	88.0	0.40
d-Xylose	180	174	140	41.7	93.5	0.54
d-Raffinose	120	532	256	41.8	0	0

found that in most cases pig erythrocytes too are permeable to glucose. Kolotilova (1937) showed (see also Kolotilova and Engel'hardt, 1937) that the speed of penetration of glucose into human and rabbit erythrocytes differs sharply. With glycolysis excluded by fluoride or iodoacetate, the diffusion of sugar out of Ringer's solution containing 0.2 per cent sugar into human blood elements finished in 10–15 min. In this period the sugar content in the cell reached 80 per cent of the external concentration.

These writers established that glucose, galactose, arabinose and xylose penetrate rabbit erythrocytes fairly quickly. Figures 35 and 36 show the

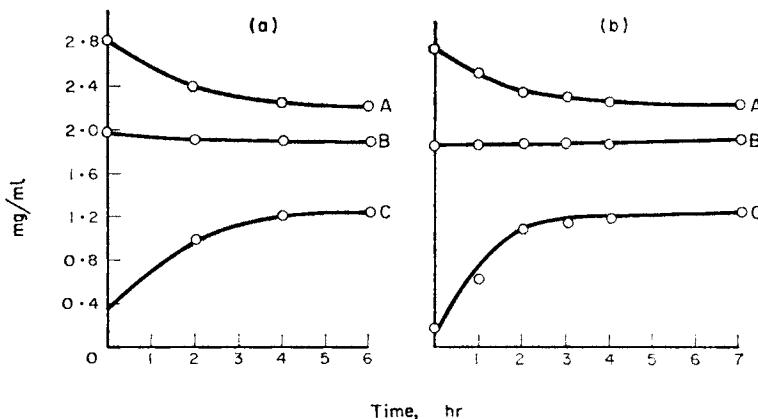


FIG. 35. The absorption of glucose by rabbit erythrocytes with time in the presence of enzymatic poisons (in mg/ml) (from Kolotilova and Engel'gardt, 1937).

a—fluoride test; b—iodoacetate test; A—sugar in the liquid part (in the medium), B—sugar in the suspension, C—sugar in the erythrocytes.

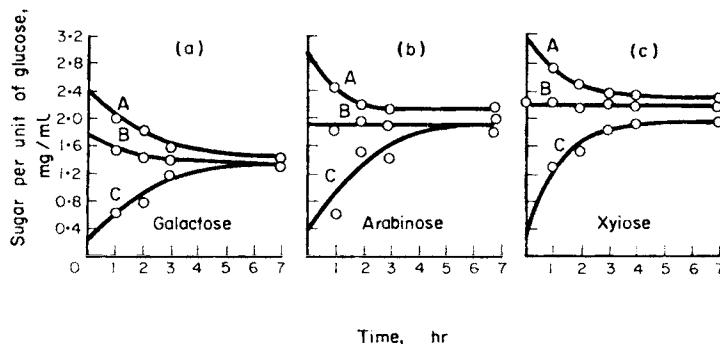


FIG. 36. The absorption of non-fermentable sugars by rabbit erythrocytes with time (sugar in glucose units, in mg/ml) (from Kolotilova and Engel'gardt, 1937).

A—sugar in the liquid part (in the medium), B—sugar in the suspension, C—sugar in the erythrocytes; a—galactose, b—arabinose, c—xylose.

absorption of sugars by erythrocytes and the excess concentration in the medium with time. They show that diffusion equilibrium between the sugars in the surrounding medium and those penetrating the erythrocytes is reached in 2–5 hr. The curves show that at the moment of equilibrium the sugar concentrations inside and outside the cell are not equal. Thus, the glucose concentration in the erythrocytes at diffusion equilibrium is half that in the medium, i.e.,  $Q = 0.5$ . For galactose, arabinose and xylose  $Q$  is significantly higher, reaching 0.75. In the same paper Kolotilova

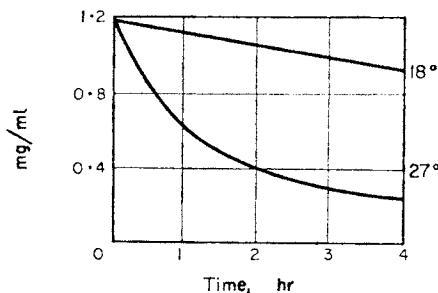


FIG. 37. The washing of sugar out of rabbit erythrocytes with time at various temperatures (in mg/ml) (from Kolotilova and Engel'gardt, 1937).

and Engel'gardt established the reversibility of the process of the absorption of carbohydrates by erythrocytes: the sugar that has penetrated the blood cells comes out at the same speed if the cells are placed in a sugarless medium (Fig. 37).

Similar results were obtained by Helmreich and Cori (1957) in experiments with rats, into whose blood arabinose, xylose and raffinose were introduced. According to these writers, the last does not penetrate erythrocytes (Table 18b).

Höber (1912) showed that, in dogs, glucose was not evenly distributed between the red cells and plasma and that the disparity was increased when the blood glucose was artificially raised or when adrenaline was given.

Shcherbatskaya (1939) carefully studied the distribution of glucose between the formed elements and the plasma of the blood of dogs *in vivo*. 16–18 hr after a meal the arterial blood contained 83–85 mg per cent glucose, 57 mg per cent being in the plasma and 26 mg per cent in the erythrocytes. Thirty min after the introduction of 50 g of glucose *per os* into a dog, 141 mg per cent was found in its blood, 99 mg per cent in the plasma and 42 mg per cent in the blood cells. The subcutaneous introduction of adrenaline was also observed to be followed by an increase in the sugar content of the blood—1 hr afterwards 158 mg per cent glucose was found in the blood, 118 mg per cent in the plasma and 40 mg per cent in the erythrocytes. When insulin was injected, the reverse phenomenon was observed; the sugar content in the plasma and the erythrocytes was lowered. These results indicate that the sugar in dog blood

is distributed between the formed elements and the plasma approximately in the ratio 1:2. This ratio remains almost constant for fairly considerable variations in the sugar concentration in the blood.

Only fragmentary information is to be found in the literature about the distribution of disaccharides between erythrocytes and the surrounding medium. From the point of view of the membrane theory, it is completely impossible for disaccharides to penetrate the formed elements of the blood for a wide range of animal and plant cells, with the possible exception of yeast cells only (Gellhorn, 1929; Höber, 1945; Rubinshtein, 1947, *et al.*). Koltilova (1937), using chemical methods, likewise failed to detect any penetration of sucrose into rabbit erythrocytes. However, the experiments of Keth and Power (1937) and Vorob'yev (1939), showed that there can be no doubt about the permeability of human erythrocytes for sucrose, both *in vitro* and *in vivo*.

Keth and Power introduced sucrose intravenously into human blood, 1 g per kg weight, and, using entirely reliable methods, determined the amount of this sugar in plasma and blood erythrocytes taken at various intervals from the veins. Thirty-seven min after the introduction of the sucrose they found 603 mg per cent in the plasma and 26 mg per cent in the erythrocytes;

TABLE 19. THE AMOUNT OF SUCROSE IN THE PLASMA AND ERYTHROCYTES  
OF STORED HUMAN BLOOD AFTER VARIOUS PERIODS OF PRESERVATION  
(from Vorob'yev, 1939)

Subject of test	Sucrose concentration (%)		
	On 6th day	On 15th day	On 25th day
Plasma	6.27	6.10	5.99
Erythrocytes	3.04	1.90	2.00
Plasma	6.29	5.89	6.08
Erythrocytes	3.37	3.40	2.22

after 1 hr 37 min, 327 mg per cent in the plasma, 49 mg per cent in the erythrocytes; after 3 hr 37 min, 152 mg per cent in the plasma and 24 mg per cent in the erythrocytes; after 6 hr 37 min, 52 mg per cent in the plasma and 18 mg per cent in the erythrocytes. Similar results were obtained from *in vitro* experiments. The writers concluded from these experiments that human erythrocytes are permeable to sucrose.

The experiments of Vorob'yev (1939) with the storage of human blood point convincingly to the same conclusion (Table 19).

Calculating the sucrose concentration in the water of the plasma and of the erythrocytes from the data of this table (taking the data from the 15th day), we obtain: 6.97 per cent in the plasma, 4.75 per cent in the erythro-

cytes, hence  $Q = 0.69$ , i.e., the sugar concentration in the erythrocytes is one third less than in the plasma.

The erythrocytes of some animals are evidently permeable not only to monosaccharides and disaccharides, but also to polysaccharides as well, which is in direct contradiction to the conclusions of the membrane theory.

Karayev and Efendiyeva (1940) in experiments with cats showed that, on the introduction of sugar into the blood whether *in vitro* or *in vivo*, the sugar moves out of the plasma into the formed elements, coming out of them upon a reduction in the concentration in the plasma. In experiments on whole animals these authors introduced sugar into the blood in large amounts. The concentration in the plasma rose sharply, but in the erythrocytes remained almost at its previous level. The authors explained this phenomenon by the synthesis of glycogen in the erythrocytes.

Increase in the glycogen in the plasma and the formed elements when loaded with sugar was observed by Gaibov (1948).

Karayev and Efendiyeva (see also Gadzhiev, Karayev *et al.*, 1947) do not admit the possibility of the accumulation of carbohydrates in the plasma because of the escape of glycogen from the erythrocytes, while Kochneva (1935) in experiments on angiostomised dogs observed that during the intake of glucose by the intestines sugar goes into the erythrocytes and glycogen comes out of them. These data demonstrate the permeability of erythrocytes not only for monosaccharides but also for polysaccharides.

There is very little glycogen in the plasma compared with in the erythrocytes (Genkin, 1938, 1939a, b; Soldatenkov, 1952). In conditions of carbohydrate deprivation or sugar loading the amount in the plasma changes. Because glycogen is not formed in the blood plasma, this also indicates that the cells of the organism are permeable for this substance. It is possible, however, that the increase of glycogen in the plasma occurs as a result of the decomposition of the formed elements of the blood (see Gadzhiev, Karayev *et al.*, 1947).

We may summarise the work quoted above as follows.

1. The erythrocytes of a wide variety of animals and humans are permeable for sugars, in particular for pentoses and hexoses. Human erythrocytes and those of some animals are permeable for disaccharides and polysaccharides as well. This conclusion sharply contradicts the membrane theory concept of erythrocytes as osmometers, because the reduction in volume of the cells observed in sugar solutions is accompanied by the penetration of these substances in large amounts into the blood corpuscles.

2. The sugar concentration within the erythrocyte in the state of diffusion equilibrium on the exclusion of glycolysis is not equal to its concentration in the surrounding fluid—in the great majority of cases the sugar concentration in the erythrocytes is less than in the medium. This is in fundamental contradiction to the ideas of Hill (1930, 1935) and other writers, according to which the erythrocytes contain almost all their water in the form of free solvent.

What are the reasons why, when one and the same sugar is distributed in conditions of diffusion equilibrium and there is no conversion by fermentation, the coefficients of the distribution of the sugar between the erythrocytes and the plasma of various types of animals are found, as can be seen from the literature cited above, to vary in magnitude, and why do various sugars in experiments on one and the same object also yield different values of  $Q$ ?

To obtain an explanation I carried out a series of experiments in which I studied the distribution of galactose between the erythrocytes of rabbits and the medium (Troshin, 1951 b). The purpose of this work was to clarify whether

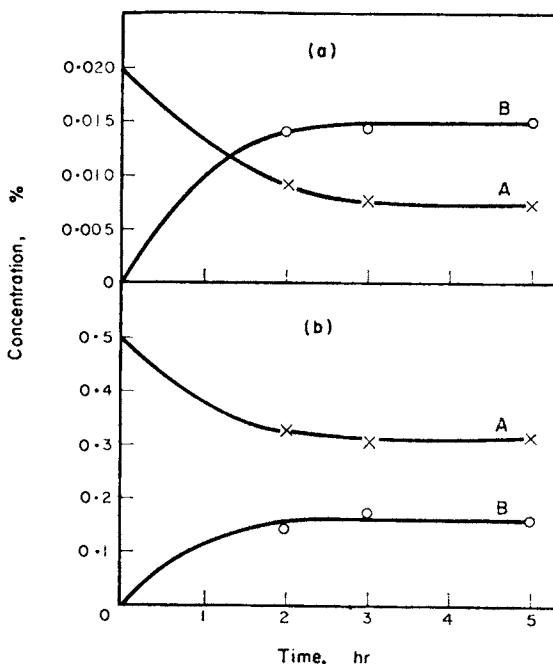


FIG. 38. The galactose in the medium and its absorption by rabbit erythrocytes with time (in per cent).

a—initial concentration in the medium 0.02 per cent; b—initial concentration in the medium 0.5 per cent; A—curves of the diminution of the sugar in the medium, B—curves of the absorption of sugar by the erythrocytes.

the sugar distribution in this case obeys the same law as the distribution of sugars between coacervates and their equilibrated solution. The sugar solutions in which the erythrocytes were placed were prepared from Ringer's solution. To avoid serious dehydration of the cells at high sugar concentrations the sodium chloride concentration in the Ringer's solution was reduced. The amount of sugar penetrating the erythrocytes was calculated from the

diminution of the amount in the medium. The volume of the erythrocytes was measured by means of haematocrits. The galactose concentration in the solution before the immersion of the erythrocytes and after their sedimentation by centrifugation was determined by the Hagedorn-Jensen method. All the experiments were carried out at constant temperature ( $37^\circ$ ).

To achieve the purpose of the work it was necessary to study the sugar distribution not at any one concentration, but at a whole series of concentrations.

In one group of experiments the time of the onset of diffusion equilibrium between the galactose penetrating the erythrocytes and the galactose in the surrounding medium was measured. These experiments showed that diffusion equilibrium is attained in 3–3·5 hr when the initial galactose concentration in the medium is equal to 0·5 and 0·02 per cent (Fig. 38).

When the equilibrium concentration of galactose in the medium is equal to 0·001 per cent, its concentration (in relation to the water) in the erythrocytes is almost two times greater, while it is halved when the equilibrium concentration in the medium is equal to 0·32 per cent.

TABLE 20. THE AMOUNT OF ADSORBED AND DISSOLVED GALACTOSE  
IN RABBIT ERYTHROCYTES AT VARIOUS GALACTOSE CONCENTRATIONS IN THE MEDIUM  
(in g per 100 ml water)

Expt. No.	Amount of water in erythrocytes (%)	Galactose concentration		Amount of total galactose in the erythrocytes		$Q = \frac{C_c}{C_s}$
		in the medium ( $C_s$ ) g/100 ml	in the erythrocytes ( $C_c$ ) g/100 ml	dissolved $C = C_s K$	adsorbed $A = C_c - C$	
1	65	0·003	0·011	0·001	0·010	3·67
2	66	0·007	0·015	0·003	0·012	2·14
3	65	0·015	0·026	0·006	0·020	1·74
4	65	0·032	0·041	0·013	0·028	1·29
5	64	0·071	0·056	0·029	0·027	0·77
6	64	0·148	0·087	0·060	0·027	0·58
7	65	0·322	0·159	0·132	0·027	0·47

The next group of experiments was performed with intermediate initial galactose concentrations to determine the relation between the percentage absorption of sugar by the erythrocytes and the concentration in the external equilibrium liquid. These experiments lasted 4 hr in all cases. In this time, as can be seen from Fig. 38, complete diffusion equilibrium is attained between the sugar in the erythrocytes and the sugar in the surrounding liquid. The results of these experiments are given in Table 20.

In the second column of the table is shown the water content of the erythrocytes. The results obtained show that the experimental method was successful in establishing a constant water content in the erythrocytes in spite of considerable variations in the sugar concentration in the surrounding liquid. This can be judged from the fact that the volume of the blood cells at the end of the experiment, as measured by means of haematocrits, remained the same as in the control batches; consequently, their water content must be the same as in the control. (The water content in the control erythrocytes was taken as 65 per cent of their total volume.)

The third column shows the concentrations of galactose in the medium and the fourth the sugar concentrations in the erythrocytes calculated per 100 ml of cell water. In the last column we give the ratios ( $Q$ ) of the sugar concentrations in the erythrocytes ( $C_e$ ) to the concentrations in the medium ( $C_s$ ).

The experimental results in this table indicate that, first, as the galactose concentration in the surrounding medium increases, the percentage absorption by the erythrocytes decreases and, second, the sugar concentration in the formed elements of the blood, calculated in relation to the volume of the water in them, can be both less and more than the concentration of this non-electrolyte in the external equilibrated liquid. Thus, if the equilibrium galactose concentration in the medium ( $C_s$ ) is 0·003 per cent, then the amount in the erythrocytes ( $C_e$ ) is 0·011 per cent, i.e., 3·67 times more than in the medium ( $Q = 3\cdot67$ ). If the sugar concentration in the erythrocytes is calculated not in relation to the cell water but for the whole volume of the cells, then we find under these conditions that the galactose concentration is about 0·0072 per cent, i.e., 2·4 times more than in the surrounding medium. When the galactose concentration in the medium is 0·032 per cent, the galactose content in the erythrocytes is 29 per cent greater ( $Q = 1\cdot29$ ), and, on the other hand, when  $C_s = 0\cdot322$  per cent, it is now reduced to about half and, in relation to the total volume of the erythrocytes, a third.

The relation between the absorption of galactose by the erythrocytes and the concentration in the medium is shown well by the curve of the change in  $Q$  with changing  $C_s$  (Fig. 39). If the galactose were not adsorbed by the erythrocyte colloids and its solubility in the water of these cells were the same as in the water of the surrounding liquid, we should obtain a value of  $Q$  equal to unity over the whole range of concentrations tried (the dotted line parallel to the axis of abscissae). In reality, the curve of the dependence of  $Q$  on  $C_s$  first falls sharply and then over the whole range of  $C_s$  up to 0·045 per cent ( $Q > 1$ ) it approaches asymptotically some constant value. In this range of concentrations (above 0·045 per cent),  $Q < 1$ , i.e., the galactose concentration in the medium is greater than in the erythrocytes.

As can be seen from the above, this dependence of the absorption of galactose by erythrocytes is analogous to that observed in the distribution of sugars between the coacervate and its equilibrated liquid. Hence it may be

concluded that such a variation in the magnitude of  $Q$  is due to the fact that the distribution of galactose is composed of two processes—adsorption of the sugar by the erythrocyte colloids and solution in the water of the cells. In view of the facts that in the conditions of our experiments the ratio of the dry residue of the erythrocytes to the cellular water at all the galactose

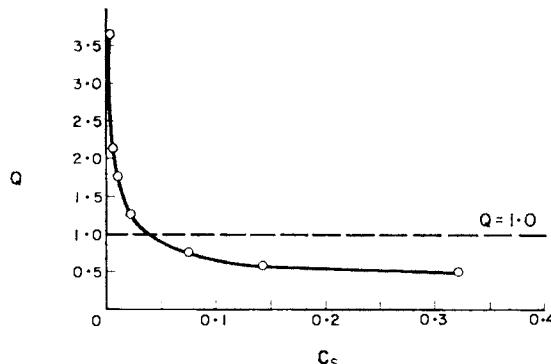


FIG. 39. The dependence of the coefficient  $Q$  on the equilibrium concentrations of galactose in the medium ( $C_s$ , in per cent) depending on its distribution between rabbit erythrocytes and medium.

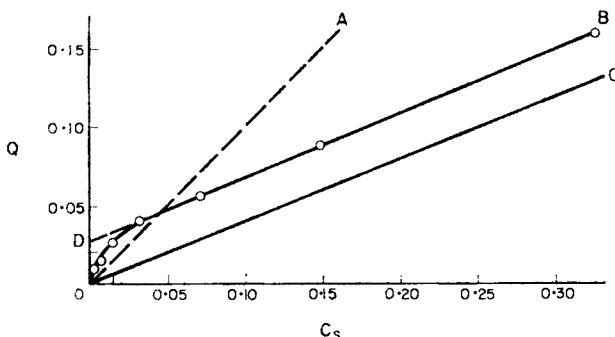


FIG. 40. The dependence of the galactose concentration in rabbit erythrocytes ( $C_s$ , in g/100 g of intracellular water) on the concentration of this sugar in the medium ( $C_s$ , in per cent).

concentrations tested was constant and the sugar concentrations were not too high, it may be supposed that the solvent properties of the protoplasm water remained practically unchanged. This makes it possible to determine graphically what proportion of the sugar in the erythrocytes is to be ascribed to adsorption and what to solution. In Fig. 40, which is constructed from the data of Table 20, the curve OB shows the dependence of the galactose concentration in the erythrocytes  $C_c$  (in per cent of the water in the cells) on the concentration of this sugar in the surrounding equilibrated liquid.

It can be seen from this graph that the limiting amount of sugar adsorbed by the erythrocytes ( $A_\infty$ ), corresponding to the sector OD, lies on the ordinate at 0.028 g per 55 g dry residue or per 100 ml cell water. Galactose is considerably less soluble in the water of the erythrocytes than in the water of the medium. The position of the straight line OC, parallel to DB, indicates that the coefficient for the distribution of the sugar between the water of the erythrocytes and the water of the medium  $K = 0.42$ , i.e., the galactose is 58 per cent less soluble in the water of the erythrocytes than in the water of the surrounding medium (at all the concentrations of the sugar tested).

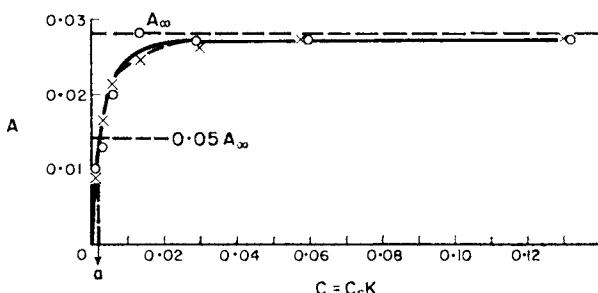


FIG. 41. The isotherm of the adsorption of galactose by rabbit erythrocytes. Along the abscissae are plotted the galactose concentrations in the water of the erythrocytes (in per cent), along the ordinate axis, the amount of adsorbed sugar (in g/55 g dry residue or 100 g cellular water).

Initially the galactose distribution curve OB runs considerably above the straight line OA, and up to  $C_s = 0.045$  per cent the amount of adsorbed sugar in the cell covers up the deficiency caused by the lowered solubility of the sugar in the protoplasm water. Further on the curve OB becomes a straight line running below the line OA. This points to the fact that the adsorption of the non-electrolyte has reached its limit and that there is no longer enough to compensate for the galactose deficiency due to its lowered solubility in the erythrocyte water.

Table 20 shows the experimentally obtained and calculated amounts of dissolved and adsorbed galactose. It shows the magnitude of the adsorbed part of the sugar in the cell compared with the dissolved part at small concentrations in the medium and the decrease in the adsorbed part with increasing concentration.

In Fig. 41 we give the isotherm of the adsorption of galactose by the colloids of the erythrocytes. It looks like a typical adsorption curve obeying Langmuir's Law. The data of Table 20 are shown in this figure by the small circles, while the values calculated according to Langmuir's formula (with coefficients  $A_\infty = 0.028$  and  $a = 0.002$ ) are shown by the crosses. The coincidence of the two sets of values for experiments of this kind must be considered to be satisfactory.

Many workers who have studied the distribution of sugars between erythrocytes and the medium have noted that with increasing sugar concentration in the medium its percentage absorption by the cells decreases (Masing, 1914a; Ege, 1920c, 1921b; Häusler, 1925a, b; Woodhaus and Pickworth, 1932, etc.). My experiments yield results in full agreement with the observations of these writers.

Some writers (for example, Kolotilova, 1937; Rubinshtein, 1947), relying on the experiments of Höber and Memmesheimer (1923) the depression by sugars of the penetration of vital dyes into erythrocytes, explain this in terms of the contraction of cell membranes at high sugar concentrations, which makes them less permeable for sugars. This explanation must be considered incorrect. The presence of a membrane, which is more or less easily permeable for sugar, can affect only the speed of attaining diffusion equilibrium, but cannot in any way influence the level of the sugar content in the erythrocytes if this sugar is not fermented by these cells. In my experiments, however, I found a high percentage of absorption of galactose at both high and low concentrations in conditions of diffusion equilibrium.

Other workers, who found in conditions of diffusion equilibrium a sugar concentration in the cells 30–40 per cent less than in the surrounding liquid, assert that in these cases the sugar is only adsorbed on the surface of the cells and does not penetrate them (Ege, 1921b; Svedberg, 1933, etc.). If, however,  $Q > 0.80$ , then, in their opinion, the sugar penetrates the erythrocytes and is dissolved in their water in the same way as in the surrounding medium, as follows from Hill's ideas about the state of the water in the protoplasm. My results argue against such an explanation. If the sugar were adsorbed on the surface and did not penetrate the erythrocytes, we should obtain a distribution curve OB in Fig. 40 which should correspond to Langmuir's formula or the empirical formula of Freundlich. In fact it does not correspond even approximately to these formulae. Further, if the sugar were only adsorbed on the surface of the cells and did not penetrate inside them, then the process of absorption of sugar by the cells would finish in a few seconds, whereas it actually takes several hours.

The majority of those who have studied the distribution of sugars between erythrocytes and the plasma or some artificial salt medium have found, as we have seen,  $Q$  to be equal to or less than unity. In most cases  $Q$  was about 0.5. Some writers explain this phenomenon by the presence in the cells of up to 50 per cent bound water (Ponder, 1940; Heilbrunn, 1952, etc.), and others by a change in the properties of the membrane.

My results show that  $Q$  can be both greater and considerably less than unity. In the case of the distribution of some non-fermentable sugars this depends on their concentration in the medium, their solubility in the erythrocytes and the adsorptional (or chemical) activity of the cells with respect to these sugars.

The information given in Fig. 38 shows that diffusion equilibrium is attained in approximately the same time at both high and low sugar concentrations and that at low concentrations the erythrocytes absorb a higher percentage of the total amount in the medium. This indicates that the permeability of erythrocytes at low sugar concentrations is greater than at high, in other words, "the permeability coefficient  $P$ " should be higher at low concentrations and lower at high concentrations.

This phenomenon has been observed by many workers in erythrocyte experiments (Ege, 1920b, c; Bjering, 1932; Klinghoffer, 1935, 1940; Bang and Ørskov, 1937; Wilbrandt, 1938b; Meldahl and Ørskov, 1940; Wilbrandt, Guensberg and Lauener, 1947; LeFevre, 1948; LeFevre and Davies, 1951; LeFevre and LeFevre, 1952; Widdas, 1953a, 1954b, etc.).

At present a number of writers, on the basis of results obtained chiefly by the osmotic method, conclude that the mechanism of the penetration into the erythrocytes of humans and some animals of hexoses and many other non-electrolytes is more complicated than simple diffusion.

It is supposed that there are in the cell membrane special carriers which regulate the passage of non-electrolytes through the membrane. The molecules of non-electrolyte, before passing into or out of the cell, form complexes with these carriers and are transported through the membrane in this form (LeFevre *et al.*, 1948, 1951, 1952, 1953, 1954, 1955; Wilbrandt, 1950, 1954, 1956; Wilbrandt and Rosenberg, 1950, 1951; Rosenberg and Wilbrandt, 1952, 1955, 1957; Wilbrandt, Frei and Rosenberg, 1956; Widdas, 1953a, b, 1954a, b, c; Bowyer, 1954, 1957; Bowyer and Widdas, 1955, 1956a, c, 1957, 1958; Park *et al.*, 1956, and others).

This form of transport must include three stages: the formation of the complex of non-electrolyte with carrier on one side of the membrane, the displacement of this complex in the membrane and its decomposition into the initial components on the other side of the membrane. Some writers suppose that enzymes participate in the formation and decomposition of the complexes at least of some hexoses with the carriers (LeFevre, 1948, 1955; Rosenberg and Wilbrandt, 1952, 1955; Bowyer and Widdas, 1958, and others).

Thus, Rosenberg and Wilbrandt (1952) put forward a very complex scheme for the penetration of substances into the cell and their exit from the cell. They suppose that the transport of substances through the cell membrane can be achieved through an enzymatically regulated transfer system. It is shown that there are three components in the mechanism of this transfer: two enzymes, one on the outer, the other on the inner, surface of the membrane, and a membrane carrier (or carriers). The enzymes cause the substrate to combine with the carrier, adopting temporarily a form dissolved in the matter of the membrane. In this form the substrate becomes transportable and diffuses through the membrane. On analysing the materials relating to the permeability of human erythrocytes for glucose, these workers came to

the conclusion that the transport of glucose into the erythrocyte is regulated by one enzyme system and the transport out by another.

In the opinion of some writers, such a mechanism for permeability permits the active transport of many sugars through the membrane of human erythrocytes (the diffusion of sugars against the concentration gradient or their displacement with greater speed than is possible with diffusion alone). Thus, Le Fevre and Davies (1951) found that aldoses (*d*-glucose, *d*-galactose, *d*-mannose, *d*-xylose and *l*-arabinose) penetrate human erythrocytes by active transport, but ketoses (*l*-sorbose and *d*-fructose) penetrate passively. The same conclusion was reached by Widdas (1954a). The hypothesis of membrane carriers was earlier suggested by Widdas (1951, 1952) to explain the transfer of glucose between mother and embryo in the sheep.

According to the membrane carrier hypothesis, the speed of penetration of substances into cells should depend in some definite way on "their affinity" to the carrier (Le Fevre and Davies, 1951; Widdas, 1952, 1954a; Wilbrandt, 1956, and others).

Thus Kozawa (1914), placing a number of sugars in order of diminishing speed of penetration into human erythrocytes at room temperature, obtained the following series: arabinose > xylose > galactose > mannose > sorbose > dextrose > levulose.

However, Wilbrandt (1938b) produced a different series: xylose > arabinose, mannose > galactose > dextrose > sorbose > levulose (the last being very much slower).

The difference between these two series is explained by the fact that the first writer took high sugar concentrations, the second low, so that the degree of saturation of the carriers with the various sugars differed, because the sugars differ in their "affinities" for the membrane carriers. The osmotic experiments of Le Fevre and Davies (1951) place the sugars in the following series in order of diminishing "affinity" to the carriers: dextrose > mannose > galactose > xylose > arabinose > sorbose > levulose. The ketoses have less affinity than the aldoses (Widdas, 1954a).

Starting from the above, Wilbrandt (1956) produced the following equation reflecting the dependence of the speed of transport of substances across the cell membrane on their concentration and "affinity" to the carriers:

$$V = DC \frac{K(S_1 - S_2)}{(S_1 + K)(S_2 + K)},$$

where  $V$  is the speed of penetration (amount of substrate passing in unit time through unit surface area of the cell),  $D$  the diffusion constant of the complex of substrate and carrier in the membrane,  $C$  the total carrier concentration,  $K$  the dissociation constant of the carrier and substrate complex,  $S_1$  and  $S_2$  the concentrations of the substrate inside and outside the cell.

At small substrate concentrations in the medium, when  $S_1, S_2 \ll K$ , the

speed of transport is inversely proportional to the dissociation constant  $K$ :

$$V = \frac{DC}{K} (S_1 - S_2),$$

while at high concentrations, when  $S_1, S_2 \gg K$ , the speed of transport is directly proportional to  $K$ :

$$V = DCK \frac{(S_1 - S_2)}{S_1 S_2}.$$

In fact, Wilbrandt found that at low concentrations sugars are to be put in one order in respect of their speed of penetration into erythrocytes, and at high concentrations in the reverse. Thus, at a sugar concentration in the medium of 0.066 M, the series is as follows: (fructose) < sorbose < arabinose < galactose < mannose (xylose) < glucose, while at 1.5 M it is: glucose < mannose < galactose < (fructose) < (xylose) < arabinose < < sorbose (both series in order of increasing speed of penetration).

It should be remarked, however, that some writers (Peters and Van Slyke, 1946, Mawe, 1956, and others) view the penetration of sugars into erythrocytes as a purely diffusional process obeying Fick's law. They assert that there are no grounds for supposing the existence of membrane carriers and an active transport system to carry carbohydrates into blood cells. They explain the deviations from Fick's law found by the above writers by assumed errors or mistakes in method.

Britton (1957) studied the speed of penetration of radioactive glucose into human erythrocytes in relation to concentration. The speed of penetration into the erythrocytes and of exit from the cells proved to be identical both at 1200 and at 2400 mg per 100 ml. From this he concludes that the speed of glucose exchange is not depressed (decreased) at high concentrations. It was found that the speed with which glucose passed through the cell membrane at a concentration of 2400 mg per 100 ml was equal to 0.04 isotonic units per sec. This is close to the value found earlier by Widdas (0.01, Widdas, 1953 a, b, 1954a).

On the basis of our own results above, we suppose that the greater permeability of erythrocytes and other cells for carbohydrates at low concentrations is due to the fact that in these conditions the greater part of the non-electrolyte absorbed by the cells is adsorbed (or chemically bound). In these conditions a steep concentration gradient between the cells and the medium can persist for a long time, so that the cell is more permeable at low concentrations than at high. It also follows from these results that the diffusion of sugars into or out of erythrocytes in all cases occurs along the concentration gradient.

*Muscle fibres.* The basic source of energy for the work performed by muscles is the splitting of carbohydrates. As this biochemical process takes place within the muscle fibre, sugar must penetrate the muscle.

However, Overton (1902a, b) insisted on the complete impermeability of muscle fibres for all sugars (pentoses, hexoses and disaccharides). He supposed that glucose can penetrate muscle fibres only in the form of some of its derivatives. The basis of Overton's assertion was that in hypertonic solutions of sugars prepared in Ringer's solution or a physiological solution the muscles decrease in volume up to a certain point, after which the volume remains constant as long as the muscles remain in the solution and are still alive. Such views are held by many workers even to-day.

However, Vandervael (1934) observed that the muscles of mammals, in contrast to those of amphibians, swell very much in an isotonic sugar solution, which, in his opinion, indicates that the sugar penetrates the muscle fibres.

The results of Trimble and Carey (1931) deserve our attention. They determined the amount of fermenting sugar in the blood, muscles and skin in healthy people and in diabetics. They obtained their experimental material at a surgical clinic. First they determined the "total sugar", then the "apparent sugar" and finally found the "true sugar" from the difference between the first and second. Their results were as follows (in mg per 100 g live tissue, mean of 7-14 experiments):

	Blood	Skin	Muscles
<b>Healthy people</b>			
Total sugar	126	69	61
Apparent sugar	10	12	33
True sugar	98	56	28
<b>Diabetics</b>			
Total sugar	238	163	83
Apparent sugar	14	19	33
True sugar	226	144	51

These results show that the sugar concentration in the muscles is in all cases less than in the skin and considerably less than in the blood.

If we consider that the normal sugar concentration in human blood plasma is about 100 mg per cent (Somogyi, 1928; Ege and Roche, 1930, etc.), we see that the skin contains two times, and the muscles three times, less than the plasma.

In diabetics, as can be seen very well from the table, there are, corresponding to the higher sugar concentration in the blood, higher concentrations in the skin and the muscles.

Cori, Closs and Cori (1933) studied the distribution of fermenting sugar between the plasma and the muscles (heart, diaphragm and various skeletal muscles) of rats, rabbits and frogs. They studied both the normal sugar level

and that found when sugar was introduced into the blood of the animal. In all cases it was found that the mean sugar concentration in the muscles was about 30 per cent of that in the blood plasma. It is interesting that a 30 min tetanus of the muscles produced by stimulation of the nerves resulted in about a two- or three-fold increase in the coefficient of the distribution of the sugar between the stimulated muscles and the plasma.

The chemical method has been used by many workers to study the permeability of muscles for sugars. Their results leave no room for doubt about the ability of these substances to penetrate muscle fibres. Thus, Schulze (1927) put frog sartorius muscles for thirty minutes in a 4·5 per cent glucose solution and found that the sugar penetrates the muscle, its concentration reaching 36·1 per cent of that in the surrounding medium in this period. It is true that the writer concluded that the glucose penetrates only the extracellular spaces. But this conclusion is erroneous, because the extracellular spaces cannot make up 36 per cent of the muscle volume. As will be shown below, they amount to about a fourth this.

Further, Eggerton (1935) studied the penetration of glucose into frog muscles and came to the conclusion that this, in his words the "fundamental nourishment of the muscles", does not penetrate the muscle fibres. He immersed isolated frog muscles (*gastrocnemius* and *semi-tendinosus*) in a 1–2 per cent glucose solution made up in Ringer's solution and determined the amount of sugar in the muscle and the surrounding solution over 10 and more hours. The experiments were carried out at a temperature of + 2–3°. Taking into account Hill's supposition (1930) that almost all the water of the muscle fibres is ordinary solvent, he expected equality of glucose concentration in the medium and the muscles. However, the concentration in the muscles turned out to be 16–27 per cent of the external concentration. This low level in the muscle cannot, as the writer points out, be explained by decomposition of the sugar occurring simultaneously with its penetration into the muscle fibre, because the total amount of glucose (in the muscles and the medium) remained constant throughout the experiment (up to 18 hr).

In hypertonic solutions of Ringer's solution muscle absorbs more glucose—up to 45 per cent of the sugar concentration in the medium. In the state of rigor mortis the muscles contain some 75–80 per cent of the concentration in the solution, i.e. the sugar in this case is distributed equally between the water of the muscle and medium.

In Eggerton's opinion, glucose penetrates only the extracellular spaces of the muscles and damaged muscle fibres but fails completely to penetrate intact muscle fibres. A series of experiments done by him with the foot muscles of *midia* argues against this conclusion. Glucose penetrates these muscles without difficulty, the concentration reaching up to 45–65 per cent of the external. It is difficult to suppose that in this case the extracellular space occupies about half of the whole muscle.

The permeability of muscles fibres has been studied in detail by numerous

workers attempting to explain the mechanism of the action of insulin on carbohydrate metabolism. There are at present different points of view on this question (see Stadie, 1954).

Thus, in a series of articles Levine and his colleagues (Levine, Goldstein, Klein and Huddlestun, 1949; Levine, Goldstein, Huddlestun and Klein, 1950; Goldstein, Mendel and Levine, 1952; Goldstein, Henry, Huddlestun and Levine, 1953; Goldstein, Mullick, Huddlestun and Levine, 1953; Levine and Goldstein, 1955) studied the effect of insulin on the speed of the reduction of the level of sugars and other substances introduced into the blood of eviscerated and nephrectomised dogs, rabbits and rats. On the basis of their results, these workers assert that only certain hexoses and pentoses penetrate muscle fibres, their penetration into the muscle cells being facilitated by the effect of the insulin. Penetration is achieved not by simple diffusion but by a special membrane transport mechanism, which is stimulated by insulin. Insulin, just like muscular work, facilitates the passage into the muscle cells only of those pentoses and hexoses which have the same configuration of hydrogen and hydroxyl groups around the first, second and third carbons as in glucose, independently of whether the sugar is utilised by the muscles or not. The effect of insulin and muscular work is seen in the case of *d*-glucose, *d*-galactose, *d*-xylose and *l*-arabinose. Of this group only *d*-glucose is utilised by the muscles. This effect of insulin and muscular work is not observed in the case of *d*-fructose, *d*-mannose, *l*-sorbose (utilised sugars), *d*-arabinose, *l*-rhamnose (non-utilisable sugars). The effect of muscular work, as is shown by these authors, is harmonious in nature and occurs independently of insulin. According to their results, insulin does not facilitate the penetration into muscular fibres of urea, sucrose, creatinine and *d*-sorbitol. Similar conclusions were reached by Drury and Wick (1951, 1952; Wick and Drury, 1951a, 1951b, 1953a, b) using carbohydrates and monohydric alcohols labelled with radioactive carbon in *in vivo* experiments with rabbits.

The views of Levine and his colleagues set out above are shared by many other workers at the present time. Various suggestions are put forward about the nature of the membrane carriers and their specific interaction with carbohydrates. In this connection much work has been done on the effect on the speed of sugar penetration into muscle cells of various hormones, enzymatic poisons and other factors (Park, Bornstein and Post, 1955; Park and Johnson, 1955; Randle, 1956; Park, Johnson, Wright and Batsel, 1957; Morgan and Park, 1957; Randle and Smith, 1958a, b; Sacks and Smith, 1958; Morgan, Randle and Regen, 1959; Wright, 1959; Morgan, Post and Park, 1961; Randle, 1961, and others). We shall return later to discuss the results published in these works.

In one study Park and his colleagues (Park, Johnson, Wright and Batsel, 1957), using eviscerated and nephrectomised rats, introduced various sugars and mannitol into the blood for 2 hr at constant speed. They found that in this time the mannitol took up only 20 per cent of the volume of the diaphragm,

22 per cent of the heart and 16 per cent of the gastrocnemius muscles. They took these amounts of "mannitol space" as representing the extracellular space in the tissue, it being considered that mannitol does not penetrate muscle fibres. The tissue spaces of *d*-fructose, *d*-mannose, *l*-arabinose, *d*-ribose and *d*-glucose were close to these sizes. On this basis the writers concluded that, in the absence of insulin, the above mentioned sugars do not generally penetrate muscle fibres. In the presence of insulin they do enter muscle fibres and their distribution coefficient in this case is raised some two to three times. They explain this insulin effect by the action of insulin on the membrane of the muscle fibre.

These conclusions are contradicted by the results of other workers. Thus, Fisher and Lindsay (1956), in experiments with the perfusion of isolated rat hearts, showed that muscle fibres in the absence of insulin is permeable for glucose and galactose alike. In the presence of insulin, heart muscle absorbs more glucose and galactose: the higher the sugar concentration, the more sugar penetrates the muscle fibres. Further, these workers showed that the speed of penetration of galactose into muscle does not change linearly with change in its concentration in the perfusing liquid. The presence in solution of glucose slows the penetration of galactose into muscle cells, and in these conditions the utilisation of glucose by the heart is decreased. The writers concluded that insulin principally increases the accessibility of cell water for the penetration into it of glucose and similar sugars.

In the last few years Cori has made a detailed laboratory study of the permeability of muscle fibres and the cells of other organs and tissues of rats for pentoses, hexoses and certain derivatives of the latter. Thus, it has been shown (Helmreich and Cori, 1957) that on the introduction into the blood of nephrectomised rats of a series of pentoses equilibrium is established between the blood plasma and the tissues in 2 hr; here the pentose concentration in the cells, calculated in relation to the intracellular water, was about half that in the blood plasma. The cells of different tissues vary in the speed of penetration of pentoses into them. In decreasing order, the series is as follows: liver, blood cells, muscle. In order of decreasing speed of penetration into gastrocnemius muscle the pentoses form the following series: *d*-xylose, *e*-xylose, *d*-arabinose, *d*-ribose, *l*-arabinose.

After the introduction of insulin into the organism and with muscular work the distribution coefficient of each of the pentoses between the water of the muscle fibres and the blood plasma is considerably increased. The increase is especially large in the case of muscular work. The introduction into the blood even of large amounts of glucose or 3-methylglucose does not depress the penetration into muscle fibre of pentoses and galactose either in normal conditions or on the simultaneous introduction of insulin.

These workers found that raffinose does not penetrate muscle fibre but is distributed between the extracellular space of the muscles and blood plasma. However, they showed that the inulin space of these muscles is less

than the raffinose and sucrose spaces. Hence it follows that sucrose and raffinose enter not only the extracellular spaces but penetrate, probably, in small quantities into the muscle fibres as well.

Elsewhere (Kipnis and Cori, 1957) in experiments on isolated rat diaphragms it was shown that *d*-xylose quickly penetrates muscle fibres, a linear relation being observed between the rate of penetration and the concentration of xylose in the medium. In experiments with "damaged" diaphragms (with ribs and central tendons removed) diffusion equilibrium was achieved in 30 min, in experiments with "intact" diaphragms in 1 hr and *in vivo* in 2 hr. In all these cases the coefficient of the distribution of xylose between the water of the muscle fibres and the surrounding solution reached 0.50–0.55. This coefficient remained constant in magnitude over a wide range of xylose concentrations in the surrounding medium. The addition to the medium of insulin caused a two to threefold increase in the speed of penetration of xylose into the muscle fibres and raised the distribution coefficient from 0.50 to 0.80. The addition of glucose had no effect on the speed of penetration of xylose into the muscle fibres.

In subsequent work Cori and his colleagues studied the dynamics of the penetration of glucose into the muscle fibres of the gastrocnemius muscles and diaphragm of rats *in vivo* (Kipnis, Helmreich and Cori, 1959) and of 2-deoxyglucose into the muscle fibres of rat diaphragms (Kipnis and Cori, 1959). On penetration into the muscle fibres, these substances are under certain conditions found in them as such, in the free form. It depends on the ratio of the speed of penetration and the speed of phosphorylation in the sarcoplasm.

On the basis of these results Cori and his colleagues reached the conclusion that muscular work and insulin act in such a way that the intracellular space available for the penetration of sugar is increased, which should lead to an increase in the speed of penetration into the cell. Further, these factors increase the speed of phosphorylation of glucose and 2-deoxyglucose and also the speed of utilisation of other carbohydrates. This should also result in an increase in the speed of penetration into the cell. The penetration of pentoses into cells is, in the opinion of these writers, a purely diffusional process. They express doubts about these effects of muscular work and insulin possibly being due to a change in the properties of the cell membrane.

The recent work of Norman and his colleagues (Norman, Menozzi, Reid, Lester and Hechter, 1959) is of great interest. They studied the mechanism of the distribution of sugars between the muscle fibres of isolated rat diaphragms and Krebs–Ringer solution. The experiments were performed on hemi-diaphragms (muscle fibres cut during the removal of the bones of the thorax) and on intact (whole) diaphragms. The distribution of *d*-galactose, *d*-xylose and *L*-xylose was studied. The extracellular space of the diaphragms was determined by the distribution of mannitol, sucrose and inulin. It is generally considered that these substances do not penetrate muscle fibres

but fill only the interstitial spaces. The basic results of this work are set out in Table 21.

The data in this table show that, if inulin shows the true extracellular space, then all the sugars and mannitol penetrate the muscle fibres, both damaged and intact, both in the presence of insulin and in its absence. The only difference consists in this, that in conditions of diffusion equilibrium the above non-electrolytes have different distribution coefficients. It is noted in this work that the distribution coefficient of the non-electrolytes tested remains practically constant over a wide range of concentrations. Summing up, the authors conclude that the cell water (of the total water in the muscle about

TABLE 21. THE DISTRIBUTION OF SUGARS BETWEEN ISOLATED RAT DIAPHRAGMS AND KREBS-RINGER SOLUTION

The sugar space (in per cent of wet weight of tissue) is indicated in which the concentration of non-electrolyte is equal to its external concentration (from Norman, Menozzi, Reid, Lester and Hechter, 1959).

Name of non-electrolyte	With insulin (+) without insulin (-)	Length of incubation (min)					
		7.5	15	30	60	90	120
(a) Hemi-diaphragms							
<i>d</i> -Galactose	+	50 ± 2	64 ± 3	75 ± 1	80 ± 2	82 ± 2	80 ± 2
	-	38 ± 2	53 ± 2	61 ± 1	74 ± 2	77 ± 2	78 ± 2
<i>d</i> -Xylose	+	51 ± 2	65 ± 2	77 ± 2	81 ± 1	80 ± 1	81 ± 2
	-	38 ± 2	50 ± 2	65 ± 2	75 ± 2	77 ± 1	80 ± 2
<i>l</i> -Xylose	+	39 ± 1	48 ± 3		64 ± 1	68 ± 1	
	-	39 ± 1	47 ± 2		60 ± 2	65 ± 2	71 ± 2
Mannitol	+		38 ± 1	46 ± 2	57 ± 4		70 ± 2
	-		38 ± 1	46 ± 2	57 ± 4	69 ± 2	70 ± 2
Sucrose	+	26 ± 1	37 ± 1	42 ± 1	46 ± 3	47 ± 3	51 ± 3
	-	26 ± 1	37 ± 1	42 ± 1	45 ± 3	46 ± 3	50 ± 3
Inulin	+		22 ± 1	26 ± 1	26 ± 1		29 ± 1
	-		22 ± 1	26 ± 1	26 ± 1	27 ± 1	29 ± 1
(b) Intact (undamaged) diaphragms							
<i>d</i> -Galactose	+	22 ± 0.6	39 ± 2.1	51 ± 2	63 ± 2	62 ± 4	64 ± 3
	-		25 ± 2.1	33 ± 1	37 ± 1.1	37 ± 2	39 ± 2
<i>d</i> -Xylose	+		38 ± 0.3	49 ± 2	65 ± 1		61 ± 1
	-		29 ± 3.0	35 ± 2	37 ± 1	35 ± 1	41 ± 1
<i>l</i> -Xylose	+		25 ± 0.4	30 ± 1	46 ± 1	50 ± 1	57 ± 2
	-		25 ± 0.4	28 ± 0.2	37 ± 1	34 ± 1	44 ± 1
Mannitol	+		23 ± 2	25 ± 3	26 ± 1		31 ± 1
	-		23 ± 2	25 ± 3	28 ± 1	31 ± 1	31 ± 1
Sucrose	+	15 ± 0.3	20 ± 1	22 ± 0.4	23 ± 0.4		29 ± 1
	-	15 ± 0.4	19 ± 1	22 ± 1.0	23 ± 0.4	30 ± 1	30 ± 1
Inulin	+		13 ± 0.4		15 ± 0.4	18 ± 1	19 ± 0.2
	-		13 ± 0.3		15 ± 0.4	18 ± 1	19 ± 0.2

80 per cent of the wet weight of the tissue) is divided into three aqueous regions which differ in their availability for the solution in them of sugars. One aqueous region is equal to 25 per cent of the total water. It is available for *d*-galactose, *d*-xylose and *l*-xylose. In the presence of insulin the water region available for these sugars is equal to 45–50 per cent, while the remaining 25–30 per cent of the total water is not generally available to these sugars. During a long incubation, so the authors suppose, the intracellular barriers are damaged and sucrose begins to penetrate the muscle fibres. In damaged muscle fibres (hemi-diaphragms) they distinguish two large aqueous regions. One is available to all sugars, including sucrose as well, the other is not. Monosaccharides and mannitol diffuse into this region at various speeds: mannitol, *l*-xylose, *d*-xylose, *d*-galactose (in order of increasing speed). In this case insulin affects only the speed of diffusion of the monosaccharides, but not their distribution coefficient. Data are also presented in this work which indicate that the uptake of glucose by the diaphragm depends on the concentration in the medium. Insulin approximately doubles this process. There is a parallel increase in the rate of synthesis of glycogen.

A careful study of the permeability for sugars of the skeletal muscles of frogs has been made by Kamnev (1938). For his experiments he took sugars that are not fermented by muscle—sucrose and galactose. Frog gastrocnemius muscles were placed in 1 per cent or 4 per cent solutions of sucrose and a 2 per cent solution of galactose made up in Ringer's solution. The sugars in the medium were determined at intervals and the concentration in the muscles calculated from the diminution of the sugar in the medium. The sugar concentration in the muscles was calculated in grams per 100 g of water held in the muscle tissue in these conditions. A parallel study was made of the penetration of the sugars into muscles killed in alcohol. The results, which are given in Fig. 42, show that first, frog skeletal muscle is fairly easily permeable for galactose and sucrose, second, the final diffusion equilibrium between the sugar of the medium and that penetrating the muscle is established in the first 3–4 hr, remaining substantially unchanged for a further 24 hr, and third, the sugar concentration in the muscles, calculated in relation to the muscle water, reaches, at the moment of diffusion equilibrium, 42·1 per cent of the external concentration in the case of galactose (initial concentration 2 per cent), 32·7 per cent and 28·4 per cent for sucrose (initial concentrations 1 per cent and 4 per cent respectively). These facts cannot be explained from the standpoint of the membrane theory. If, as Hill thought, this amount of sugar in the muscles is contained only in the extracellular spaces and in damaged muscle fibres, the undamaged being impermeable for sugar, why was the galactose concentration in the muscles found to be 42 per cent of the external concentration and the sucrose 33 per cent for one concentration and 28 per cent for the other—the higher? It is also unintelligible that the muscle fibres should be damaged only in the first part of the experiment and not suffer further damage in the course of 24 hr.

Kamnev concluded that sucrose and galactose penetrate muscle fibres and that the amount of sugar in the fibres is determined not by a membrane mechanism, but by the solubility of these substances in the muscle sarcoplasm, which he thinks of as a phase with different solvent power from the water of the surrounding solution. Dead muscle, in his opinion, loses the specific phase properties of live muscle, which explains the equalisation of the concentrations in dead muscle and the medium.

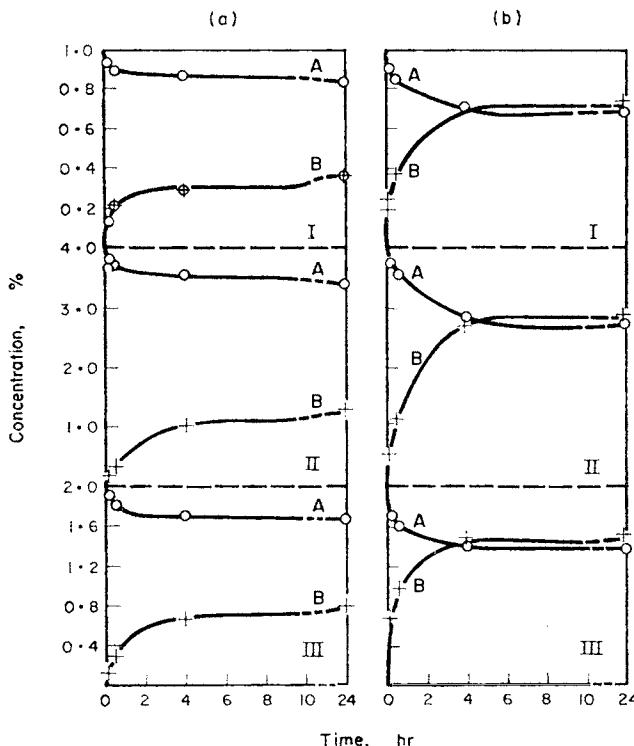


FIG. 42. The passage of sugar into live and dead skeletal muscles of frogs from the surrounding medium with time (after Kamnev, 1938).

a—live muscles, b—dead muscles; A—diminution of the sugar in the medium, B—absorption of sugar by the muscles. Initial sugar concentrations in the solution: I—1 per cent sucrose solution; II—4 per cent sucrose solution, III—2 per cent galactose solution.

Krogh and Lindberg (1944), experimenting with isolated functional frog hearts, found that the muscle fibres are permeable for glucose and sucrose besides sodium ions. In their experiments they passed Ringer's solution through the hearts after replacing part of the sodium chloride in the solution by glucose or sucrose. Recently, Barclay (Barclay *et al.*, 1959) experiment-

ing with the perfusion of isolated rat hearts showed convincingly that the muscle fibres of this organ are permeable for sucrose.

Gzhatskii and Vandokanti (1947) found that on the subcutaneous injection of glucose into frogs the rate of glycogen synthesis in the muscle and liver increases: the more glucose is introduced, the higher their glycogen content. This also indicates that glucose can penetrate muscle fibres and liver cells.

The Hungarian physiologists Hetenyi, Issekutz *et al.* (1953) established that sugars injected into dogs intravenously are absorbed by the skeletal muscles and return to the blood under certain conditions.

Skeletal muscle is probably permeable not only for monosaccharides and disaccharides but also for polysaccharides. From their experiments with angiostomised dogs Fedorov and Namayatisheva (1935), London, Kochneva and Rivosh (1936), Kochneva (1938a, b) and others suppose that muscle is permeable for glycogen because the latter is in certain conditions released.

In the opinion of some workers (for example, Gellhorn, 1929), smooth muscles have permeability for sugars of a completely different kind from skeletal muscles. The available literature does not, however, permit us to see here any difference in principle. It indicates rather that there is similarity in principle in the permeability phenomena of smooth and transversely striated muscle. Thus, Meigs (1914) in the case of the adductor of the mollusc *Venus mercenaria* observed that cane sugar passed very quickly into the muscle fibres. This muscle survives in 30 per cent sugar solution for 44 hr without losing its excitability. It absorbs so much sugar out of the 30 per cent cane sugar solution in  $\frac{1}{2}$  and 17 hr that the sugar concentration in the muscle water reaches 17.5 per cent and 62.5 per cent respectively of the external concentration. In view of this Meigs supposed that smooth muscle fibres do not have any semi-permeable membrane.

The writer sought to explain the inequality of the sugar concentrations in the medium and the muscle at diffusion equilibrium by the presence in the muscles of 38 per cent water bound by the colloids which takes no part in the solution of the substances penetrating the muscle, while the remaining 62 per cent is normal solvent.

Analogous results were obtained by Heymann (1925) in experiments with frog stomach muscles. Smooth muscles were observed to absorb 1.82 g per 100 g of muscle in 4–5 hr from an isotonic cane sugar solution (7.5 per cent sugar plus 0.02 per cent calcium chloride). In an isotonic solution without the calcium chloride the muscles absorbed somewhat more—2.0 per cent. During the period indicated the sugar concentration in the water of the muscles reached 50 per cent of the external. This writer also sought to explain this phenomenon by the presence of bound water in the muscle.

Recently Bozler and Lavine (1958) showed that fibres of the stomach muscles of the frog *Rana pipiens* are easily permeable for fructose and sucrose. They established that the sucrose space of this muscle is 37 per cent. If the inulin space is taken into account (the extracellular space of the

muscle), it is found that only 20 per cent of the cell water is available for the solution of sucrose. This percentage is higher for fructose. The authors indicate that with diminishing molecular weight of the non-electrolyte the "non-solvent space" of the muscle also decreases. This contradicts the theory that there is in the muscle a certain amount of "bound water" which will not generally dissolve any substance. Elsewhere (Bozler, 1932) the same laws of the distribution of sucrose and a number of other non-electrolytes were established in experiments with the sartorius and stomach muscles of frogs.

Thus, chemical methods have shown that both transversely striated and smooth muscle fibres are permeable not only for monosaccharides but also for disaccharides.

Here too, as in the case of erythrocytes, we do not observe equalisation of the sugar concentrations in the medium and the muscles; further, as is shown by the experiments of Kamnev and other workers, the distribution coefficient varies for different sugars and different concentrations in conditions of diffusion equilibrium.

To determine the causes underlying this phenomenon I studied the distribution between the gastrocnemius muscles of frogs and the medium of non-fermentable sugars; arabinose, galactose and sucrose (Troshin, 1948a).

The experiments were performed as follows. Frog gastrocnemius muscles were prepared and carefully separated from the tendons so as not to damage the muscle fibres and then immersed for 30 min or 2 hr in frequently changed Ringer's solution. After this the muscles were dried with lignin, weighed on a torsion balance and placed in salt solutions containing a known amount of sugar. The sugar solutions were used in Ringer's solution which was made isotonic by the addition of suitable amounts of sodium chloride. The total weight of the muscles did not exceed 350–400 mg. In 5 hr, by which time diffusion equilibrium had certainly been reached (see Fig. 42), the muscles were taken out of the solutions, dried with lignin and again weighed, while the concentration of the sugar remaining in the surrounding equilibrated liquid was also determined. The sugar concentration in the muscles was calculated from the loss in the medium.

The sugar concentration in the muscles  $C_c$  was calculated in per cent of the water in them. The water content of the muscles before immersion in the sugar solutions was 80 per cent of the total muscle weight (see Hill, 1930, 1935; Fenn, 1936; Kamnev, 1938, etc.). Further, the sugar concentration in the water of the muscle fibres was also calculated. The amount of extracellular water of the muscles was taken as 10 per cent of the muscle weight (on extra-cellular spaces, see Ch. VIII, para. 1). The sugar concentration was then determined by the formula:

$$C_c = \frac{aV - a_1(V + m + 0.1m - m_1)}{0.7 - (m - m_1)},$$

where  $a$  is the percentage of sugar in the medium before the experiment,

$a_1$  that after the experiment (after the removal of the muscles from the solution),  $V$  the volume of the surrounding liquid at the beginning of the experiment in ml,  $m$  the weight of the muscles (in grams) at the beginning of the experiment,  $m_1$  the weight at the end.

The results of these experiments are summarised in Table 22, where only average values are given, made up at each sugar concentration from three to sixteen experiments.

TABLE 22. THE DISTRIBUTION OF SUGARS BETWEEN FROG MUSCLES AND MEDIUM  
(Concentrations of sugars in per cent)

Name of sugar	Sugar concentration			$Q = \frac{C_c}{C_s}$	Sugar concentration in muscle fibres, calculated from the formula $C_c = C_s K + A$	Difference between the experimental (col.4) and theoretical (col.6) concentrations			
	in the medium $C_s$	in the muscles							
		per total tissue water	per intracellular water $C_c$						
Arabinose	0.13	0.15	0.17	1.31	0.15	+ 0.02			
	0.32	0.24	0.25	0.78	0.23	+ 0.02			
	0.68	0.36	0.32	0.47	0.38	- 0.06			
	1.40	0.76	0.67	0.47	0.69	- 0.02			
	2.63	1.39	1.22	0.46	1.22	± 0.00			
Galactose	0.025	0.021	0.022	0.88	0.028	- 0.006			
	0.050	0.032	0.028	0.56	0.036	- 0.008			
	0.56	0.29	0.26	0.46	0.20	- 0.06			
	1.28	0.57	0.50	0.39	0.43	+ 0.07			
	2.15	0.87	0.77	0.36	0.71	+ 0.06			
Sucrose	0.067	0.036	0.035	0.52	0.029	+ 0.006			
	0.75	0.29	0.24	0.32	0.23	+ 0.01			
	1.72	0.60	0.53	0.31	0.51	+ 0.02			
	2.38	0.78	0.69	0.28	0.70	- 0.01			

From the table it follows that within the limits of the sugar concentrations tried  $Q$  varies by a factor of 2-3. Thus, at an arabinose concentration in the medium of 0.13 per cent,  $Q = 1.31$ . At higher concentrations  $Q$  is less than unity. When the arabinose concentration in the medium is 2.63,  $Q = 0.46$ . This means that there is 54 per cent less sugar in the muscle fibres than in the surrounding equilibrated medium.

For all the galactose and sucrose concentrations tried  $Q$  is less than unity; but, as in the first case,  $Q$  increases considerably with decreasing sugar concentration in the surrounding liquid. This dependence of  $Q$  on the sugar concentrations in the medium is shown for all three sugars by the curves in Fig. 43. The curves form a series in which the sugars remain in the same order at all concentrations: arabinose > galactose > sucrose.

Thus at a sugar concentration  $C_s = 0.4$  per cent,  $Q$  is 0.78 for arabinose, 0.45 for galactose and 0.35 for sucrose. In every case we find the same dependence as we detected in our experiments with coacervate systems. The dependence is well represented by our previous formula:

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

Figure 44 shows the relation between the sugar concentration in the muscles ( $C_c$ ) and that in the medium ( $C_s$ ).

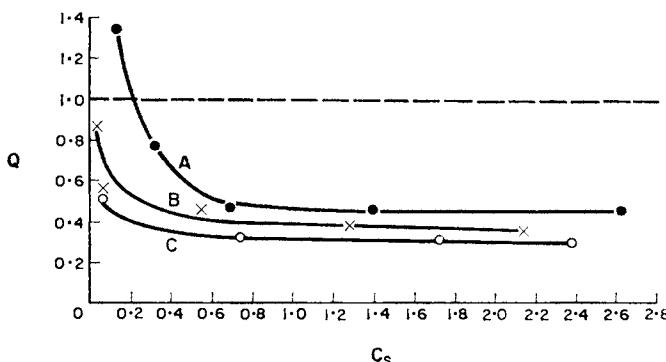


FIG. 43. The dependence of the coefficient  $Q$  on the concentration of sugars in the medium ( $C_s$ , in per cent) on the distribution of these non-electrolytes between muscles and the surrounding equilibrated solutions.

A—arabinose, B—galactose, C—sucrose.

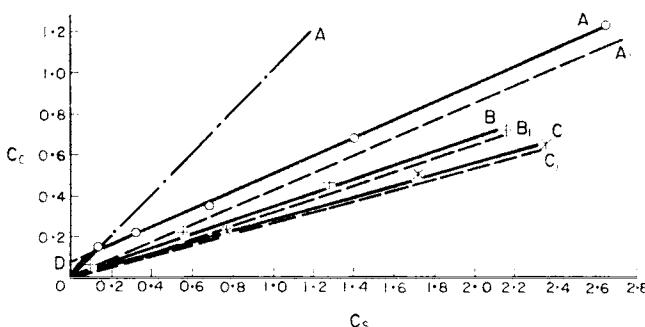


FIG. 44. The dependence of the sugar concentration in muscle fibres ( $C_c$ , in g per 100 ml of intracellular water) on their concentration in the medium ( $C_s$ , in per cent).

The curves characterise the sorption of arabinose (A), galactose (B) and sucrose (C) by muscle fibres. The lines  $A_1$ ,  $B_1$ ,  $C_1$  respectively determine the amount of these sugars in the muscles in the dissolved state.

As can be seen from the figure, within the limits of the concentrations tested the relation is linear. Consequently, within these limits of the variation of the sugar concentration in the medium, the quantity of adsorbed non-electrolyte will be constant, corresponding to the adsorption limit  $A_\infty$ . Hence the above formula becomes simply:  $C_c = C_s K + A_\infty$ .

Clearly, the sugar distribution curves in Fig. 44 must start from the point of intersection of the coordinate axes and must therefore resemble adsorption isotherms in their initial parts.

The curves OA, OB, OC in Fig. 44 show the arabinose, galactose and sucrose distributions respectively. If the straight parts of the distribution curves are extended, the intercept with the ordinate is numerically equal to  $A_\infty$ , which is denoted for arabinose by OD in the figure. For the other sugars, this portion will be correspondingly smaller, as the curves indicate. The straight line OA<sub>1</sub>, starting from the origin of co-ordinates and parallel to DA, reflects the dependence of the concentration of arabinose dissolved in the water of the muscle fibres (C) on the concentration in the medium ( $C_s$ ), which is:  $C = C_s K$ .

This dependence for galactose and sucrose is shown by the lines OB<sub>1</sub> and OC<sub>1</sub> respectively. The coefficient  $K$  can easily be calculated from the slope of these lines.

Thus it was found that:  $A_\infty$  is 0·09 for arabinose, 0·02 for galactose and 0·01 for sucrose (in grams per amount of dry muscle substance corresponding to 100 ml of muscle fibre water);  $K$  is 0·43 for arabinose, 0·32 for galactose and 0·29 for sucrose.

In the sixth column of Table 22 we give the sugar concentrations in the muscle fibres calculated from our formula using these values of  $A_\infty$  and  $K$ . The coincidence of the experimental and calculated values is completely satisfactory (see column 7 of Table 22).

The experimental values of  $K$  show that, independently of the sugar concentration in the medium, arabinose is 57 per cent, galactose 68 per cent and sucrose 71 per cent less soluble in the protoplasm of muscle fibre than in the water of the surrounding medium. Consequently, if the sugars are placed in order of their solubility in the water of the muscle protoplasm and the magnitude of the limiting amount of adsorbed sugar  $A_\infty$ , the same series is obtained as when the value of  $Q$  was the criterion, that is:

$$\text{Arabinose} > \text{galactose} > \text{sucrose}.$$

It should be noted that in this order the sugars are also in order of increasing molecular weight.

Thus, our results completely coincide with those of Kamnev (1938) and certain other workers. They sharply contradict the ideas of the membrane theorists, according to whom the predominant part of the protoplasm water is a simple solvent. According to Hill (1930, 1935) there is about 95 per cent free water in muscle, according to Rubinshtein (1947) at least 80 per cent. If

if this were true, then the distribution coefficient  $K$  for all sugars independently of their concentration would be about 0.95—according to Hill (in which case a distribution curve close to the line OA in Fig. 44 would obtain)—or 0.8—according to Rubinshten. In fact, the distribution coefficients are considerably lower and differ as between one sugar and another.

Thus, the level of the absorption of sugars by muscle is determined by their lowered solubility in the sarcoplasm and adsorptional or chemical binding by the colloids of live tissue.

The literature and my own observations indicate that the lower the molecular weight of the sugar and its concentration in the medium, the higher will be the distribution coefficient  $Q$ , i.e., the greater the percentage of sugar absorbed by the muscles from the surrounding solution.

It is easy to see that the distribution of sugars between muscle and medium is completely analogous to that observed in experiments with erythrocytes and coacervates.

*Nerve and other cells.* Elliott (1946a) observed violent swelling of sections of rat brain in isotonic solutions (0.308 M) of glucose, fructose and sucrose. Swelling was also observed if the sugar concentration was four times above the isotonic. The addition to the sugar solutions of electrolytes reduced the swelling of the slices. The writer concluded from these results that brain cells are permeable for sugars under these conditions.

Park and his colleagues (Park and Johnson, 1955; Park, Johnson, Wright and Batsel, 1957) studied the permeability of brain cells for a number of sugars. Their experiments were performed with eviscerated and nephrectomised rats into whose blood various monosaccharides and mannitol were introduced. The value of the distribution coefficient between the water of the blood plasma and the tissue water was used as a measure of the penetration of the sugar into the cells. The size of the extracellular spaces was determined from the mannitol distribution. They found that *d*-galactose, *d*-mannose, *d*-xylose, *l*-arabinose and *d*-glucose penetrate brain cells, the distribution coefficient varying within the limits 0.3–0.6. According to their results, *d*-fructose and *d*-ribose completely fail to penetrate brain cells because their distribution coefficient is equal to that of mannitol. Insulin has no effect on the penetration of sugars into cells in the case of the brain.

Shanes and Berman (1955a, b) observed that sucrose-<sup>14</sup>C does not penetrate nerve fibres: it passes only into the extracellular spaces of nerves.

In experiments *in vitro* with the crystalline lens of rabbit eyes Ross (1953) found that glucose is absorbed by this tissue at a rate of 0.2, and under the effect of insulin of 0.7, milligrams per gram of tissue per hour. Galactose is apparently not absorbed by this organ in the absence of insulin, but, in the presence of the latter, it is absorbed at a rate of 0.11 mg/g/hr.

Of great interest are the experimental results of Krane and Crane (1959). They studied the accumulation of *d*-galactose in the cells of rabbit kidney cortical slices, the galactose being labelled with radioactive carbon. The slices

were incubated in Krebs-Henseleit solution with a phosphate buffer in the presence of various concentrations of labelled galactose. The average amount of water in the slices was 73·6 per cent of the wet weight of the tissue. The extracellular space of the slices was determined by the distribution of raffinose: it was 33 per cent of the wet weight of the slices. The concentration of the galactose entering the slices was calculated in relation to the intracellular water. Some of the results are given in Figs. 45, 46 and 47.

The curves in these figures show that galactose very quickly passes into the cells of the slices and accumulates in concentrations considerably exceeding the external. In the presence of enzymatic poisons and in a nitrogen atmosphere the accumulation of sugar in the cells is depressed. In these conditions the sugar concentration in the cells at equilibrium approximates to the external or is even lower than that.

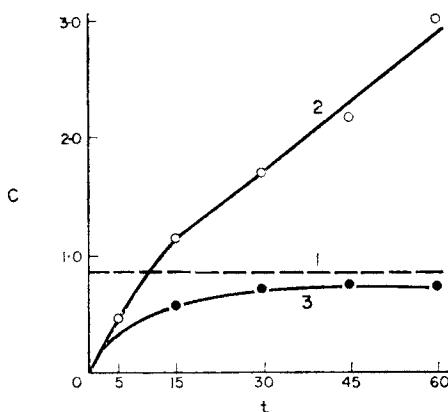


FIG. 45. The accumulation of galactose-1- $^{14}\text{C}$  in the cells of sections of the cortex of rabbit kidney (from Krane and Crane, 1959).

Ordinates—the galactose concentrations in the cells (in  $\mu\text{M}/\text{ml}$  of intracellular water), abscissae—time in minutes.

1—the concentration of galactose in the medium, 2—concentration in the cells (control), 3—concentrations in the cells in conditions when there is dinitro-o-cresol ( $7 \times 10^{-5} \text{ M}$ ) in the medium.

The authors of this paper concluded that galactose is accumulated in the cells against the concentration gradient thanks to an active transport mechanism whose operative components are located in the cell membrane. Their data obey the Michaelis-Menten rule. Enzymatic poisons and the absence of oxygen depress this membrane transport mechanism.

It seems to us, however, that these results can better be explained from the standpoint of the sorptional theory. From the point of view of this theory, the galactose accumulates in the cells because, on entering a cell along the diffusion gradient, it is bound in the protoplasm by adsorption or some

other cause. Enzymatic poisons and the replacement of oxygen by nitrogen depress this binding process; in this case the galactose passes into the cell also along the concentration gradient till diffusion equilibrium is established. When there is complete depression of the binding of sugar in the cell, diffusion equilibrium is established at a sugar concentration in the cell lower than that

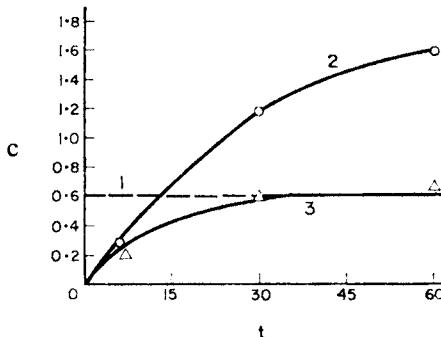


FIG. 46. The effect of the absence of oxygen on the accumulation of galactose in cells of sections of rabbit kidney cortex (from Krane and Crane, 1959).

1—concentration of galactose in the medium, 2—concentration in the cells (in  $\mu\text{M}/\text{ml}$  intracellular water) in an atmosphere of oxygen, 3—the same in an atmosphere of nitrogen.

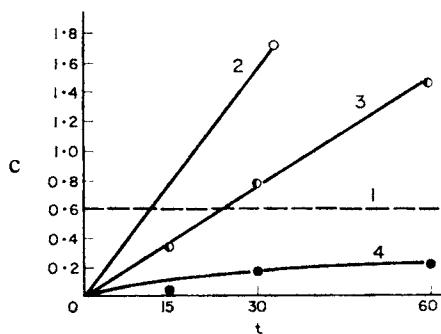


FIG. 47. The effect of phlorizin on the accumulation of galactose in the cells of sections of rabbit kidney cortex (from Krane and Crane, 1959).

1—the concentration of galactose in the medium, 2—control, 3—in the presence of phlorizin  $23 \times 10^{-4} \text{ M}$ , 4—the same at  $93 \times 10^{-4} \text{ M}$ .

in the medium. This is due to the fact that the water in the protoplasm is in a special state, the solubility in it of substances being considerably lower than their solubility in ordinary water.

In interpreting their results in terms of the hypothesis of a membrane transport mechanism the authors of the work under discussion do, however, note that it is hard to exclude the possibility of dissociational binding

of the substance accumulating in the cell with the components of the protoplasm, a remark with which one cannot but agree.

This work by Krane and Crane has been discussed in such detail because it is typical of many studies of the mechanism of the penetration of sugars into erythrocytes, muscle fibres and other cells. In most of these works, as we have seen, the facts are interpreted from the point of view of the hypothesis of membrane carriers, whereas it seems to us that these facts can better be explained within the framework of the sorptional theory.

A series of experiments by Crane and his colleagues has demonstrated the ability of the epithelial cells of the small intestine of warm-blooded animals to accumulate certain sugars. This phenomenon is connected by the authors with a mechanism of active transport of sugars through the intestinal wall during their absorption (Crane, 1960a, b; Crane and Mandelstam, 1960; McDougal, Little and Crane, 1960; Miller and Crane, 1960).

As an example, we may take another thorough study made very recently by Rickenberg and Mavio (1961). They studied the accumulation of galactose in the cells of a suspension of mouse tissue culture (L-form cells). It was found that at equilibrium, when the galactose concentration in the medium is lower than 10 mm, the sugar concentration in the cells is higher than outside (at a concentration of  $1 \times 10^{-5}$  M thirty times higher), while at a galactose concentration in the medium of 25 mm the concentration in the cells is half that in the medium. Dinitrophenol, phloretin, phlorizin, low temperatures and the presence in the medium of glucose depress the accumulation of galactose in the cells. The authors explain their results by an active transport mechanism in the cell membrane; but their results can easily be explained in the framework of the sorptional theory of cell permeability.

Interesting information on the permeability of Ehrlich ascites tumour cells for a number of sugars and their derivatives is given in a paper by Crane, Field and Cori (1957). In Table 23, which is taken from this paper, the fundamental characteristics of the substances tested are shown: they indicate variations in the speed of their penetration into the tumour cells.

It is remarked in this paper that the speed of penetration in a series of twelve sugars varies by a factor of 20, which indicates, in the opinion of the authors, the high degree of specificity of the cell membrane. The speed of penetration of sugars into the cell is strongly temperature dependent. At 20–30°  $Q_{10}$  for this process is about 4. It increases non-linearly as the temperature falls to zero, when sugar practically fails to pass into the cells. This phenomenon is also explained by a change in the specific properties of the cell membrane. Competition between sugars is observed in the speed of their passage into the cell. Five per cent serum albumin, insulin and glutamine, and likewise the presence or absence of oxygen, do not affect the speed of penetration of 3-methylglucose. The passage of sugars into cells is analogous to their exit from them. Raffinose, phlorizin and *N*-acetylglucosamine do not

TABLE 23. THE SPEED OF PENETRATION OF VARIOUS SUGARS INTO EHRLICH  
ASCITES CARCINOMA CELLS AT 20°C  
(from Crane, Field and Cori, 1957)

Name of sugar	Sugar concentration in surrounding solution (mole/l.)	K-constant of speed of penetration of sugar into cells ( $\text{min}^{-1}$ )	Initial speed of penetration of sugar into cells (mole/l. cells/min)	$K$ for sugar/ $K$ for 3-methylglucose
3-Methylglucose	0.033	0.53	0.0175	1.0
1,5-Sorbitane	0.0167	1.6	0.0267	1.5*
Galactose	0.033	0.62	0.0202	1.17
<i>D</i> -Arabinose	0.033	0.48	0.0158	0.91
Lyxose	0.033	0.46	0.0152	0.87
Xylose	0.033	0.34	0.0113	0.65
Allose	0.0167	0.34	0.0057	0.32*
<i>L</i> -Sorbose	0.033	0.14	0.0046	0.26
2-Deoxyglucose	0.033	0.12	0.0040	0.23
Glucose	0.0066	0.28	0.0019	0.22*
Glucosamine	0.033	0.074	0.0024	0.14
Ribose	0.033	0.046	0.0015	0.09
<i>D</i> -Arabinose	0.033	0.032	0.0011	0.06

\* Ratio found for constants  $K$  determined at identical concentrations of the sugar and 3-methylglucose in the surrounding liquid.

penetrate these cells, but phlorizin depresses the passage of sugars into the cells.

According to the data yielded by the osmotic experiments of Stewart (1931a), dextrose and sucrose, like erythritol and glycerol, practically do not penetrate unfertilised eggs of the sea urchin *Arbacia punctulata*. These results need to be checked by other methods, because the osmotic method, as was shown above, is not valid in this application, at least in a number of cases (see Aizenberg, 1939).

## 2. The permeability of cells for urea and its derivatives

*Erythrocytes.* Since the publication of the work of Hamburger (1889, 1891), Overton (1895), Grijns (1896) and Hedin (1898) it has been thought that erythrocytes, like other animal and plant cells too, are easily penetrated by urea (Roncato, 1923; Mond and Hoffmann, 1928b; Gellhorn, 1929; Hill, 1935; Wilbrandt, 1938; Höber, 1945; Stewart, 1931a; Jacobs, 1952; Love, 1953, etc.). If one may judge by the results of osmotic experiments, urea passes into the cells more slowly than glycol and glycerol.

Many workers have reported that urea, which is a permanent component of blood, is distributed between the formed elements and the plasma in un-

equal quantities. Thus, Wu (1922) in the blood of twenty healthy people found 17.1 mg per cent nitrogen urea in the erythrocytes and 19.3 mg per cent in the plasma, while Folin and Berglund (1922b) found an average of 11.5 mg per cent in the blood of youths: 10.3 mg per cent in the formed elements and 12.4 mg per cent in the plasma. Folin and Svedberg (1930) found somewhat less urea in the blood elements than was found by Folin and Berglund. In their opinion, the urea in the erythrocytes is in two forms, one diffusible, the other non-diffusible. The concentration of diffusible urea in the erythrocytes, calculated not in terms of the cell volume but in terms of their water content, is 75–89 per cent of the concentration of this substance in the plasma water.

Lundsgaard and Holböll (1926) found in the blood of a nephritic human 0.285–0.305 per cent urea (0.320–0.335 per cent in the plasma, 0.221–0.248 per cent in the erythrocytes). In *in vitro* experiments with the blood of healthy people they showed that urea added to the plasma is distributed between the latter and the erythrocytes with a distribution coefficient of 0.72. They concluded that the urea is distributed in different quantities between the aqueous phases of the erythrocytes and the plasma. Somewhat different conclusions were reached by Woodhaus and Pickworth (1932). In experiments with washed sheep erythrocytes they observed rapid penetration of urea into these cells. At urea concentrations of 0.33 to 5.5 per cent in the salt solutions in which the erythrocytes were suspended, the concentration of this non-electrolyte in the water of the blood corpuscles after 30 min reached 61 to 63 per cent of the external concentration.

Parpart and Shull (1935a, b), using washed cells of cattle, rabbits and dogs, also established the rapid penetration into these cells of urea from a solution made up in Ringer-Locke solution. Using a urea solution with a concentration of about 0.3 M they found that the amount of urea in the erythrocytes after 40–60 min reached a constant concentration of 107 per cent (cattle), 111 per cent (rabbit) and 104 per cent (dog) of the concentration in the surrounding medium when the urea concentration was calculated in relation to the water content of the erythrocytes. The urea, so the authors suppose, on passing into the cells is not only dissolved in their water but also adsorbed by the colloids of the live matter. Similar results were obtained by Ørskov (1946a) in experiments with human erythrocytes. Different conclusions were reached by Conway and Kane (1934): they determined the urea concentration in the blood, muscles and other tissues of the frog. Their results show the blood containing about 46 mg per cent urea, distributed in equal quantities between the aqueous phases of the plasma and the erythrocytes.

Certain urea derivatives (thiourea, methylurea and diethylurea) are found by osmotic methods to penetrate easily the erythrocytes of various kinds of animals (Mond and Hoffmann, 1928b; Gellhorn, 1929; Wilbrandt, 1938, etc.). According to Love (1953) urea penetrates the erythrocyte-like cells of

*Phoscolosoma gouldi* 4·5 times slower than ethyleneglycol, slower than thiourea, but considerably faster than tetraethylglycol and glycerol. A chemical analysis performed by Williams and Kay (1945) shows that thiourea very quickly penetrates human erythrocytes and also the erythrocytes and cells of other tissues of rats. In one hour after the introduction of urea *per os* into a human they found in the plasma 0·72 mg per cent of this substance with 1·97 mg per cent in the blood corpuscles.

On the introduction of thiourea into the blood of rats it was found that its concentration in the plasma is less than in the erythrocytes and less in the latter than in the leucocytes. The same is observed in *in vitro* experiments. Thus, blood cells contain considerably more thiourea than the plasma. The authors suppose that it is, apparently, partially bound by the proteins in the cells.

The data cited above give grounds for supposing that the distribution of urea and its derivatives between the erythrocytes and the plasma or artificial salt media is regulated by the same factors as those responsible for the distribution of sugars observed in experiments with blood elements, muscles and coacervates. It may be supposed that the solubility of urea and its derivatives in the protoplasm water is less than in the surrounding aqueous medium and that these substances vary in their ability to be adsorbed or chemically bound by the colloids of various cells. The conjunction of the above factors also gives the observed pictures of the distribution of urea and its derivatives between erythrocytes and media.

*Muscle fibres and cells of other tissues and organs.* Folin, Berglund and Derick (1924) in experiments on dogs found a considerable excess of urea concentration in the muscles over the concentration in the plasma.

Conway and Kane (1934) determined the urea content of various frog organs and obtained the following data (in mg per 100 ml of tissue water):

1st series	Blood (whole)	45 ± 2·5
	Sartorius	41 ± 2·8
	Kidney	85 ± 3·6
2nd series	Blood (whole)	53 (39–65)
	Sartorius	48 (38–52)
	Kidney	97 (76–100)
	Liver	147 (118–170).

In the opinion of these writers, urea is contained in different concentrations in the aqueous phase of the cells of all organs and tissues and the aqueous phase of the blood plasma.

Elsewhere (Conway and Fitzgerald, 1942), it was established that, on the injection of urea into the blood of mammals all the water of the skeletal muscles and the cortex of the kidneys is available for the solution of the urea and that a certain amount of this substance can be bound by the colloids of these tissues, while the water of the cortex of the (cephalic) cerebrum, the

medulla oblongata and the spinal cord is only partially available for the solution of urea. In Table 24, which is taken from this work, we show data relating to the speed of diffusion of urea into sections of various organs of a number of animals. The mean diffusion coefficient for urea for all tissues is  $8.3 \times 10^{-5} \text{ cm}^2/\text{min}$ . The coefficient for inulin for kidney slices is lower than  $3.36 \times 10^{-5} \text{ cm}^2/\text{min}$ .

TABLE 24. THE SPEED OF DIFFUSION OF UREA INTO SECTIONS  
OF VARIOUS MAMMALIAN TISSUES AT 38°C

(from Conway and Fitzgerald, 1942)

Tissue	Animals	Mean thickness of sections (mm)	Limits of varia- tion of the diffusion coeffici- ent $K(\times 10^5)$ ( $\text{cm}^2/\text{min}$ )	Mean value of $K(\times 10^5)$ ( $\text{cm}^2/\text{min}$ )
Kidney	Rabbit	0.76	0.5-10.4	5.3
	Cat	0.77	6.7-7.8	7.3
	Dog	1.14	2.7-21.2	12.3
Muscle	Rat	0.85	7.5-10.3	8.2
	Rabbit	1.11	—	7.3
Liver	Rat	1.13	7.6-13.2	11.2
	Rabbit	1.56	4.22-5.9	5.1
Cerebral cortex	Cat	1.29	—	11.8
	Rabbit	2.42	6.5-8.0	7.2

Levine and his colleagues (Levine, Goldstein, Huddlestone and Klein, 1950) concluded that on being introduced into the blood of eviscerated and nephrectomised dogs and rats urea quickly penetrates all tissues and organs and is distributed in equal concentrations in all their water. Shanes and Berman (1955a, b) found that urea in large quantities penetrates the isolated sciatic nerve of a toad. The authors suppose that all the water of the nerve is available for the solution of urea. The same conclusion was reached by Kipnis and Cori (1957) experimenting on isolated rat diaphragms.

The permeability of smooth and striated isolated frog muscles for urea and thiourea was studied by Bozler (1959). He found that the time constant for the penetration of urea into the sartorius was 15 and for exit from the muscle  $15.5 \text{ min}^{-1}$ . For stomach smooth muscle this constant was 11 and  $10 \text{ min}^{-1}$  respectively. The time constant for the entry of thiourea into the sartorius was 12 and into the stomach muscle  $6 \text{ min}^{-1}$ . The author found that the "urea" space of both muscles was considerably higher than the total space of the muscle, which indicates adsorption of part of the urea by the sarcoplasm.

The permeability for urea of striated frog muscles was studied by Eggleton (1930). He established that in 2 to 3 hr the urea concentration in the

muscle reaches a constant value (diffusion equilibrium is reached) exceeding the concentration in the surrounding salt solution. In his opinion, the urea is soluble in the water of the muscle fibres just as in the surrounding medium, the excess in the sarcoplasma being explained by its adsorption by the muscle colloids.

However, to determine the solubility of urea in the protoplasm, it is necessary to know what proportion of the urea penetrating the cell is bound by the colloids of the live tissue and what proportion is in the dissolved state. I attempted to clarify this question and to compare the mechanism of urea distribution with that of the distribution of sugars between cells and the medium. This was the more interesting because urea, as is well known, has a number of specific properties which show in its action upon live tissue and protein solutions. It is known that urea in certain concentrations very strongly increases the excitability of muscles (Troshin, 1939; Makarov, 1948 b, and others). At moderate urea concentrations the muscles are dehydrated, while, on the other hand, at higher concentrations, where the excitability of the muscles falls quickly, the muscles swell violently (Nasonov and Aizenberg, 1937). It is also known that urea causes the denaturation of cell proteins and isolated native proteins and prevents them from coagulating (Makarov, 1948b).

Urea is adsorbed in large quantities by various proteins. Thus, Pasynskii and Chernyak (1950) found that 1 g of human serum albumin can adsorb 0.36 g of urea, 1 g of human serum globulin 0.43 g, 1 g of gelatin 0.52 g and 1 g of keratin 0.08 g. Strong binding of urea in a solution of egg albumin was also observed by Weber and Versmold (1931).

I have studied the distribution of urea between frog gastrocnemius muscles and the medium (Troshin, 1954). The experimental procedure was the following. Solutions of urea were made up in Ringer's solution. The urea concentration in the surrounding medium before the immersion of the muscles and after their removal was determined by a nitrogen manometer. A control (muscle immersed in Ringer's solution without urea) was employed in each experiment to correct the results of the analysis of the experimental tests.

The method was first used to study the dynamics of the passage of urea into the muscles with time; the results of this series of experiments are given in Fig. 48. The curves A denote the change in the urea concentration in the medium and B its absorption by the muscles. The curves show that at an initial urea concentration in the medium  $C_s = 4$  per cent (Fig. 48a) and at  $C_s = 0.5$  per cent (Fig. 48c) the substance quickly penetrates the muscles: in 1½ to 3 hr diffusion equilibrium between the muscles and the external urea solution is established, there being then no change for a length of time up to 5 hr. We also see that diffusion equilibrium is attained at such a ratio of urea concentration in the muscles ( $C_c$ ) and in the medium ( $C_s$ ) that in the first case (initial urea concentration 4 per cent)  $Q < 1$ , while in the second (initial concentration 2 per cent)  $Q > 1$ . In the first case the urea concentra-

tion in the muscles calculated in relation to all the water of the tissue is 7-10 per cent less than in the medium, while in the second it is about 35 per cent more and in the third two times more. Thus we see here the same dependence of the absorption of urea by live muscles as we observed in the experiments with sugars: the lower the concentration of the substance in the medium, the relatively more it is absorbed by the cells.

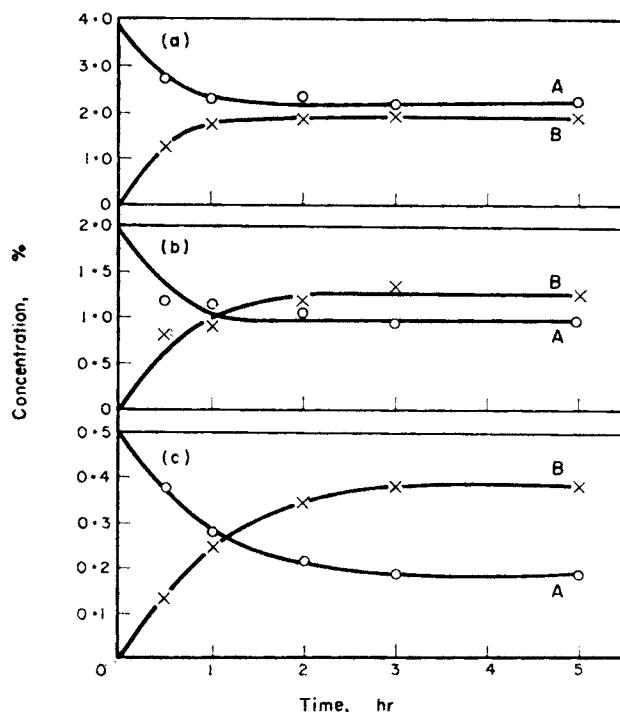


FIG. 48. The penetration of urea into frog muscles from the surrounding solution with time.

A—diminution of the urea in the medium, B—absorption of urea by the muscles; initial urea concentrations: a—4.0 per cent, b—2.0 per cent, c—0.5 per cent.

This feature of the distribution of urea between muscle and the surrounding medium was studied in more detail in another series of experiments where, in addition to the concentrations already tried, both greater and lesser concentrations were used. These experiments lasted 3 hr in all cases, a period completely sufficient for the establishment of diffusion equilibrium, as can be seen from the curves of Fig. 48. The results of this series are shown in Table 25.

Figure 49 gives the dependence of  $Q$  on  $C_s$ . Within the limits of the concentrations used the muscles absorb urea from 96 to 226 per cent of the

TABLE 25. THE DISTRIBUTION OF UREA BETWEEN FROG GASTROCNEMIUS MUSCLES AND THE SURROUNDING EQUILIBRATED LIQUID. TEMPERATURE 20-23°C

No. of Experiments	Urea concentration (%)				$Q = \frac{C_c}{C_s}$	Excitability of muscles in cm of induction coil control (experiment)		
	in the medium ( $C_s$ )		in the muscles					
	arithmetic mean	deviation from mean	per total tissue water ( $C_m$ )	per intra-cellular water ( $C_c$ )				
5	0.19	0.16-0.26	0.39	0.43	2.26	$\frac{18-22}{30-40}$		
6	0.41	0.36-0.53	0.72	0.82	2.00	$\frac{18-22}{35-36}$		
5	1.04	1.00-1.07	1.22	1.29	1.24	$\frac{18-22}{35-65}$		
6	1.63	1.52-1.75	1.75	1.80	1.10	$\frac{18-22}{35-65}$		
5	2.25	2.21-2.29	2.12	2.15	0.96	$\frac{18-22}{17-19}$		
4	2.67	2.37-2.96	4.40	4.34	1.63	$\frac{18-22}{5-10}$		

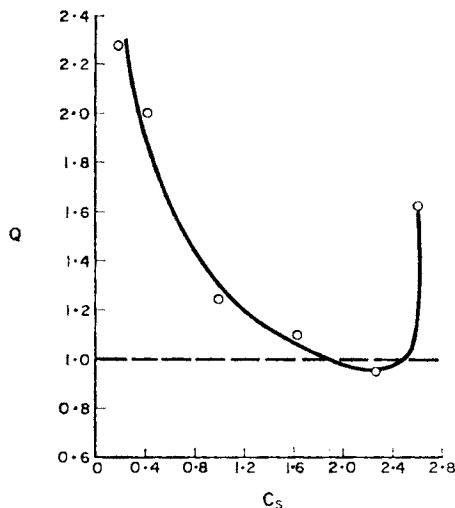


FIG. 49. The dependence of the coefficient  $Q$  on the concentration of urea in the surrounding equilibrated solution ( $C_s$ , in per cent) when urea is distributed between frog muscles and medium.

external concentration, and it is only in the region where  $C_s$  is between 2·0 and 2·4 per cent that the urea concentration in the medium and the muscle is equal or slightly less in the latter.

The results of this series of experiments are depicted in Fig. 50 in the form of a curve which reflects the dependence of the urea concentration in the muscles on the concentration in the external medium.

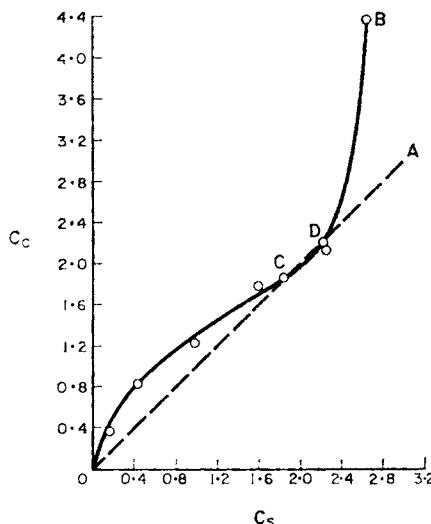


FIG. 50. The dependence of the urea concentration in frog muscle fibres ( $C_c$ , in g/100 g of intracellular water) on its concentration in the medium ( $C_s$ , in per cent) in conditions of diffusion equilibrium.

The bisector OA in this figure would reflect the dependence of  $C_c$  on  $C_s$  in the case where the solubility of urea in the water of the muscle fibres was the same as in the water of the surrounding medium and when there was also no adsorption by the muscle colloids. However, in reality the dependence of  $C_c$  on  $C_s$  is expressed by the S-shaped curve OB, which twice intersects the line OA (at the points C and D). From the origin to the point C the curve OB lies above the bisector OA ( $C_c > C_s$  and, consequently,  $Q > 1$ ); here it intersects it, then lies beneath to the point D ( $C_c < C_s$  and  $Q < 1$ ), where it again intersects and rises sharply ( $C_c > C_s$  and  $Q > 1$ ). In the region where the urea concentration in the medium  $C_s$  lies between 1·9 per cent and 2·5 per cent the muscle fibres at equilibrium contain less urea than the surrounding medium, while on both sides of this zone, on the other hand, the concentration in the fibres is greater than in the medium.

What are the factors underlying this pattern of urea distribution?

The colloids of muscle tissue strongly adsorb urea. This is convincingly demonstrated by the parts of the distribution curve OC and DB that lie

above the bisector OA. The adsorption of urea in large quantities by the colloids of live cells (muscles, erythrocytes, plant cells, etc.) is indicated in many places in the literature, as was said above. However, if the solubility of urea in the water of muscle fibres was the same as in the water of the surrounding medium, the distribution curve OB could not go beneath the bisector as it does in the sector CD (i.e.,  $C_c$  could not be less than  $C_s$ ). It would run considerably above the line OA. In any case, at  $C_s = 2.25$  per cent,  $C_c$  should be not 2.15 per cent, as it is in fact, but not less than 2.65 per cent, because even at  $C_s = 0.41$  per cent,  $C_c = 0.82$  per cent, i.e.,  $C_c$  is twice as large as  $C_s$ . But in these conditions  $C_c$  would be still larger because it is unlikely that the adsorption of urea would have reached its limit at  $C_s = 0.41$  per cent. If, as in fact happens, when  $C_s = 2.25$  per cent,  $C_c = 2.15$  per cent, i.e., the urea concentration in the muscle fibres is 4 per cent less than in the medium, this can only be, clearly, because the solubility of urea in the water of the protoplasm must be at least 30 per cent less than in the water of the surrounding medium.

At the point D the urea distribution curve again intersects the bisector and then rises sharply upwards. Thus, when  $C_s = 2.67$ , the urea concentration in the muscle fibres is 63 per cent higher than in the medium ( $Q = 1.63$ ). The shape of the curve OB in the sector DB shows that, as the urea concentration in the medium increases, the urea, on penetrating the muscle fibres, causes such changes in the sarcoplasm as are accompanied by a sharp increase in the sorptional properties of those fibres. It is probable that at this stage the muscles already suffer some damage.

In this connection it is interesting to note that the excitability of muscles in 1–2 per cent urea solution is considerably greater than that of the control muscles immersed in Ringer's solution (see Table 25). Even in a 2.67 per cent urea solution in the first 30–40 min enhanced excitability is also seen. Thereafter it falls off quickly but never drops to zero during the whole course of the experiment (3 hr).

Thus, it may be supposed that adsorption by the colloids and lowered solubility of urea in the water of the sarcoplasm explains the shape of the distribution curve for this substance.

This example of the distribution of urea shows clearly that the adsorbing power of cells and the lowered solubility of a substance in the protoplasm are not always constant. They can vary within wide limits with changes in the functional state of the cells. Especially large changes, towards both the high and the low side, can occur in the adsorbing power of the protoplasm colloids, which cause a change in the absorption of the substance by the cells from the medium. This phenomenon has been particularly well studied by Nasonov (1959) and his colleagues in experiments on the distribution of vital dyes.

In the present case it is evidently the urea itself that is the agent which, at certain concentrations, produces those changes in the sarcoplasm that lead to a sharp enhancement of the adsorptional properties of the muscle colloids.

It is probable that the solubility of urea in the water of the protoplasm is increased simultaneously. The lowering of the excitability of the muscles at high concentrations of urea shows that it acts here as a deleterious agent.

Membrane theorists suppose that the increase or decrease in the permeability of cells on excitation, narcosis or damage depends on the change in the permeability of semi-permeable cell membranes. However, such an explanation of the increased binding of urea by muscles at high concentrations is inapplicable because urea even under normal conditions very successfully penetrates a wide variety of plant and animal cells. Further, this mechanism by itself cannot possibly produce the considerable excess of the urea concentration in the muscles over that in the medium.

Thus, the lowered solubility of urea in the sarcoplasm and the binding of it by the colloids of live tissue determine the extent of its absorption by the muscles out of the medium, this extent also depending on the urea concentration in the surrounding solution. Hence it follows that the distribution of urea and sugars between cells and medium is regulated by one and the same mechanism. In this respect there is no difference in principle between them.

The literature shows that the distribution of some urea derivatives between muscles and medium is also regulated by the same sorptional factors as regulate the distribution of urea and the other non-electrolytes mentioned above. Thus, for example, Williams and Kay (1945) observed that thiourea, when introduced into the blood of rats, penetrates the cells of various tissues and organs in a few minutes. They found that when there was 6 mg per cent of thiourea in the blood, there was half as much in the muscles but two to three times more in the liver (calculated in every case per 100 ml of tissue water).

### 3. *The permeability of cells for creatinine*

*In vivo* creatinine in the blood is distributed between the formed elements and the plasma. In the blood of healthy people Wu (1922) found the following distribution: 1·24 mg per cent in the plasma and 2·48 mg per cent in the erythrocytes.

According to Folin and Svedberg (1930), human red blood cells contain creatinine in two forms: bound and diffusible. The amount of dissolved creatinine comes to 58–90 per cent of the concentration in the plasma.

According to Fenn (1936), frog skeletal muscles normally contain 1·7 mg of creatinine per kg of fresh tissue and the blood plasma 2·1 mg.

There is no question about the permeability of live cells for creatinine. Like urea, this substance is formed in the cells, then passes into the blood and is later removed from the organism as an end product of intracellular metabolism.

According to the results of Eggleton (1930), muscle fibres are freely permeable for creatinine just as they are for urea (see also Levine, Goldstein, Huddlestun and Klein, 1950).

A more detailed study of the distribution of this substance, one not foreign to the organism, as was done for urea and sugars in the experiments described above, presented itself as an interesting object of further research. The experiments were in fact done on frog gastrocnemius (Troshin, 1952).

Folin's method was used to determine the creatinine concentration in the medium before the immersion of the muscles and at the end of the experiments. The loss of creatinine from the medium determined the concentration in the muscles. Controls were also used to allow correction of the experimental results.

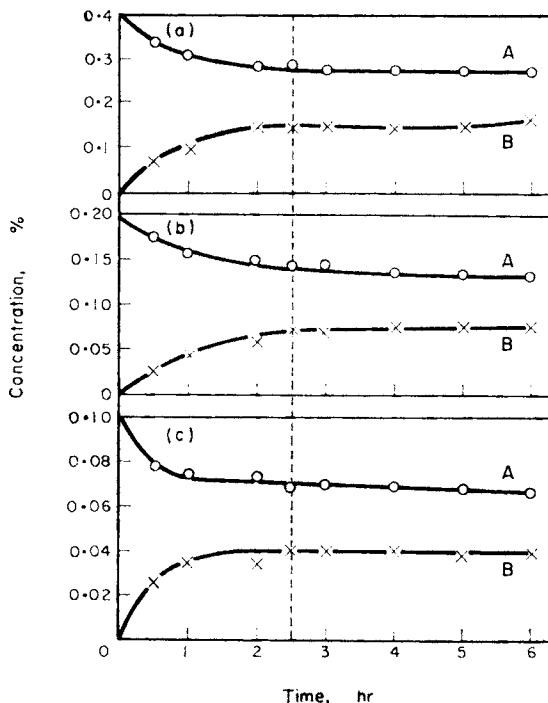


FIG. 51. The penetration of creatinine into frog muscles from the surrounding medium with time.

A—diminution of the creatinine in the medium, B—the absorption of creatinine by the muscles; initial creatinine concentrations: a—0.4 per cent, b—0.2 per cent, c—0.1 per cent.

First of all the speed was established with which diffusion equilibrium is achieved between the muscles and the surrounding Ringer's solution, which contained creatinine. The results of these experiments are given in Fig. 51. Here curves A show the loss of creatinine from the medium, curves B its passage into the muscles.

The curves A show that for all initial concentrations of creatinine (0.1 per cent, 0.2 per cent and 0.4 per cent) the concentration in the medium quickly

falls, reaching a definite level in 2–2.5 hr and remaining for a long period thereafter substantially unchanged. Correspondingly, the creatinine concentration in the muscles (curves B) grows quickly at first, reaches a definite level in 2–2.5 hr and thereafter also remains substantially unchanged. It should be noted that the creatinine concentrations used, though they are considerably above the levels normally encountered in the organism, evidently do not damage the muscles, because the threshold of excitability of the latter during the course of the whole experiment does not differ from the threshold in the controls.

Thus, this series of experiments indicates that creatinine easily penetrates skeletal muscles and that at complete diffusion equilibrium the concentrations in the muscles and the surrounding medium are not the same.

Next we studied the dependence of the creatinine concentration in the muscles on the concentration in the medium at diffusion equilibrium. Supplementary experiments with lower initial concentrations (0.05, 0.025 and 0.0125 per cent) were performed. They ran for 3 hr, which was completely adequate for the establishment of diffusion equilibrium. The results of this group of experiments are shown in Figs. 52 and 53.

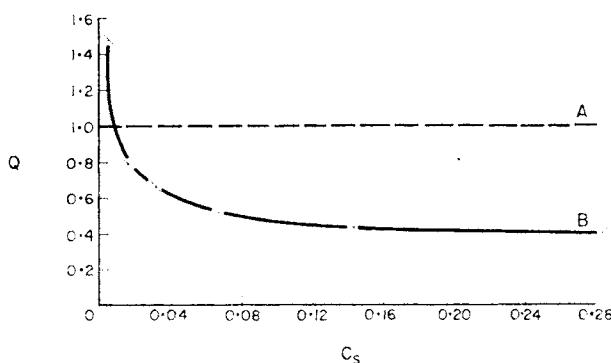


FIG. 52. The dependence of the coefficient  $Q$  on the creatinine concentration in the medium ( $C_s$ , in per cent) when creatinine is distributed between frog muscles and Ringer's solution.

The curve B in Fig. 52 shows the dependence of  $Q$  on the creatinine concentration in the surrounding equilibrated medium in which the muscles were immersed. The straight line A, parallel to the abscissa, corresponds to  $Q = 1$ . This value of  $Q$  would obtain if the creatinine concentrations in the muscle fibres and the medium were equal. However, curve B shows that when the creatinine concentration in the medium is less than 0.01 per cent, the concentration in the muscles is greater than in the medium. With increasing creatinine concentration in the medium,  $Q$  rapidly falls, gradually approaching some constant value. When  $C_s = 0.284$ ,  $Q = 0.41$ . In other words, the creatinine concentration in the muscle fibres is 59 per cent less than in the

medium, while when  $C_s = 0.006$  per cent, on the other hand, it is 50 per cent more.

This shape of the curve of the dependence of  $Q$  on  $C_s$  is evidently due to the same causes as govern the distribution of sugars and urea: adsorptional binding of the creatinine by the cell colloids and lowered solubility of the substance in the protoplasm. The point can be made convincingly by presenting the above data in the form of a graph of the dependence of  $C_c$  on  $C_s$ .

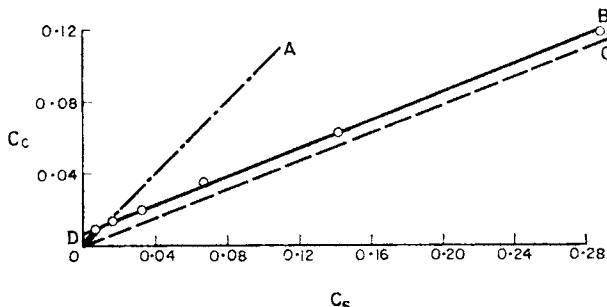


FIG. 53. The dependence of the creatinine concentration in frog muscle fibres ( $C_c$ , in g/100 ml of intracellular water) on its concentration in the surrounding liquid ( $C_s$ , in per cent).

This dependence is shown by the curve OB in Fig. 53. We see that over the whole range of concentrations used there is a linear relation between  $C_c$  and  $C_s$ , which is given by the formula:

$$C_c = C_s K + A_\infty.$$

The sector on the ordinate OD, corresponding to  $A_\infty$ , is numerically 0.001 gm per 28.6 g of dry muscle weight or per 100 g of intracellular water. This is the maximum amount of creatinine that the muscles can bind. The line OC, parallel to DB, obeys the law  $C = C_s K$ , where  $C$  is the concentration of dissolved creatinine in the muscle fibre. The coefficient of proportionality in this equation to the straight line is  $K = 0.38$ . Consequently, the solubility of creatinine in the sarcoplasm is 62 per cent less than in the water of the surrounding medium, independent of the concentration in the latter.

Thus, the literature and my own observations indicate that the distribution of creatinine between the cells and medium obeys the same law as the distribution of sugars, urea and its derivatives. At small concentrations the percentage absorption of creatinine by the cells out of the medium is greater than at high concentrations, so that the permeability of muscle fibres for creatinine increases with decreasing concentration in the medium, just as is seen in the experiments with sugars and at low concentrations in the experiments with urea.

*4. The permeability of cells for monohydric alcohols  
and certain other non-electrolytes*

On the basis of osmotic experiments, the supporters of the membrane theory assert that ethylene glycol in all cases penetrates animal and plant cells more quickly than glycerol and the latter penetrates certain cells more quickly than urea. Tetrahydric and pentahydric alcohols penetrate cells considerably more slowly than glycerol, while hexahydric alcohols already seem to be incapable of penetrating animal and plant cells (Overton, 1902 a; Wilbrandt, 1938; Höber, 1945, etc.).

Parpart and Shull (1935 a, b) established that ethylene glycol and glycerol very quickly penetrate bovine erythrocytes washed in Ringer's solution. In the experiments with ethylene glycol diffusion equilibrium was already attained in 15 min, while glycerol required 40–60 min. However, the concentrations of these substances in the aqueous phases of the cells and the surrounding solution were observed not to equalise. Thus, at a 0·1949 M concentration of ethylene glycol in the medium, the concentration in the water of the erythrocytes reached only 74 per cent of the external concentration, while at a 0·195 M concentration of glycerol in the medium the concentration in the water of the blood corpuscles reached only 67 per cent. The authors assume that glycerol and ethylene glycol are not adsorbed by the cell colloids. Rather, in their opinion, the lower concentration of these substances in the cell in comparison with the surrounding medium is to be explained by the presence in the erythrocytes of a certain amount of bound water. The amount of such water, as determined by the difference between the ethylene glycol concentration in the cell and in the medium is: in bovine erythrocytes 26 per cent, in dog 29 per cent, in human 33 per cent and in sheep 29 per cent. However, if the glycol concentration in the erythrocytes were 26 per cent lower than in the medium, then under the same conditions the glycerol concentration in these cells would be 33 per cent lower than the external. This shows that for various substances one and the same cell has different amounts of "bound water".

MacLeod and Ponder (1936) also determined the amount of "bound" water in human, rabbit, bovine and sheep erythrocytes on the basis of the distribution of ethylene glycol. At a concentration of ethylene glycol in the medium of about 0·18 M, the concentration in the water of the erythrocytes was 3 per cent higher on the average. On the basis of their experiments the writers concluded that ethylene glycol quickly penetrates erythrocytes and reaches a concentration equal to that in the medium. In contrast to the preceding workers, they concluded that there is, in general, no bound water in erythrocytes and that all the water in the cell is ordinary solvent. However, in my opinion, they had no grounds for such an assertion. Their experiments show that ethylene glycol is adsorbed by the colloids of live tissue. In so far as it is not known what proportion of the ethylene glycol in the cell is ad-

sorbed and what proportion dissolved, nothing definite can be said about the amount of bound water.

To determine the amount of "solvent water" in human erythrocytes Ørskov (1946a) studied the distribution of glycol, glycerol, malonamide, urea and glucose between these cells and serum or a 0·9 per cent solution of sodium chloride. The non-electrolyte concentration in the erythrocyte suspension was varied from 0·125 to 0·5 M. In every case it was established that at diffusion equilibrium the non-electrolyte concentration, calculated relative to the aqueous phase of the erythrocytes, was higher than in the surrounding medium. This worker supposes that the non-electrolytes used by him are adsorbed by the colloids of the erythrocytes and the serum, but to different degrees, as a result of which this inequality in the concentrations in the cells and the surrounding medium is found.

Hunter and Parpart (1938) found that 99 per cent of the water of the abdominal muscle of the frog *Rana pipiens* is available for the solution of ethylene glycol.

Results published by a number of workers (Henry, Huddleston and Levine, 1952; Wick and Drury, 1951a, b; Goldstein, Mullick, Huddleston and Levine, 1953; Morgan and Park, 1957; Park, Johnson, Wright and Batsel, 1957; Morgan, Randle and Regen, 1959) show that mannitol as well as sorbitol does not penetrate the cells of animal tissues but fills only the extra-cellular spaces. This conclusion is based on the fact that the distribution coefficients of mannitol and sorbitol between the tissue and the plasma or some artificial salt solution differs little from the distribution coefficient of inulin and remains unchanged by insulin or muscular work. However, Fisher and Lindsay (1956), perfusing isolated rats' hearts, found that the inulin space is considerably less than the sorbitol space. This indicates that sorbitol penetrates the muscle fibres. The inulin space for rat diaphragms is also considerably less than the mannitol space, which also points to the permeability of muscle fibres to this substance (Norman *et al.*, 1959). According to Bozler (1959), the sartorius and stomach muscle of frogs are permeable to erythritol and sorbitol.

Love (1953) used the haemolytic method proposed by Jacobs (1934) to determine the permeability constants for a number of non-electrolytes, including ethylene glycol and glycerol, for the erythrocyte-like cells (cells from coelomic fluid) of *Phascolosoma gouldi*. The results are given in Table 26, where the permeability constants of these substances for other cells are also given for comparison. On the basis of these results, Love came to the conclusion that the speed of penetration of the non-electrolytes into cells is determined by their lipoid solubility and molecular volume. Similar results from experiments with guinea pigs are given by Parpart (1936), who also used the haemolytic method to determine the penetration of alcohols and sugars into cells. Several workers (Stewart, 1931a; Lucké, Ricca and Hartline, 1936, and others) have used the osmotic method to determine the per-

meability of the eggs of marine invertebrates (*Arbacia*, *Cumingia*, *Chaetopterus*) for a number of alcohols and their derivatives. They obtained a typical Overton permeability series.

TABLE 26. PERMEABILITY CONSTANTS OF VARIOUS NON-ELECTROLYTES  
FOR VARIOUS TYPES OF CELLS  
(from Love, 1953)

Substance	Time in seconds for 50% haemolysis of "erythrocytes" of phascolosoma at 25°; conc. = 1M	Permeability constant $P \times 10^4$ (M/cm <sup>2</sup> of surface/hr at a concentration difference between the cells and medium of 1M)			
		Erythrocytes of phascolosoma	Eggs of sea urchin	Cells of algae characeae	Ox erythrocytes
Formamide	158	59		770 <sup>(2)</sup>	1760
Acetamide	124	95	345 <sup>(1)</sup>	530 <sup>(2)</sup>	1760
Propionamide	73	374	850 <sup>(1)</sup>	1300 <sup>(2)</sup>	685
Butyramide	53	2460	2200 <sup>(1)</sup>	1700 <sup>(2)</sup>	980
Ethylene glycol	185	44	240 <sup>(3)</sup>	430 <sup>(2)</sup>	58 <sup>(3)</sup>
Diethylene glycol	281	21	150 <sup>(3)</sup>		27 <sup>(3)</sup>
Triethylene glycol	637	7.1			12 <sup>(3)</sup>
Tetraethylene glycol	1573	2.5			
Urea	552	9.6		40 <sup>(2)</sup>	6500 <sup>(3)</sup>
Thiourea	423	12.1		77 <sup>(2)</sup>	7 <sup>(3)</sup>
Glycerol	< 14400	> 0.025	3 <sup>(1)</sup>	7 <sup>(1)</sup>	0.7 <sup>(3)</sup>
Monacetine	1137	3.7		160 <sup>(1)</sup>	41 <sup>(3)</sup>
Diacetone	248	26.8		800 <sup>(1)</sup>	375 <sup>(3)</sup>
1,3-Propanediol	186	44	260 <sup>(3)</sup>		36 <sup>(3)</sup>
1,2-Propanediol	145	69	460 <sup>(3)</sup>	870 <sup>(1)</sup>	118 <sup>(3)</sup>
Water	49.2	72	22 <sup>(4)</sup> for entry 18 <sup>(5)</sup> for exit		400 <sup>(3)</sup>

Note: data taken from: <sup>(1)</sup> Jacobs and Stewart (1932); <sup>(2)</sup> Collander and Bärlund (1933); <sup>(3)</sup> Jacobs (1952); <sup>(4)</sup> Jacobs (1932); <sup>(5)</sup> Lucké, Hartline and McCutcheon (1931).

Lucké and his colleagues (Lucké, Hempling and Makler, 1956), using the osmotic method, determined the speed of penetration of various alcohols into various cell components of lymphoma and their normal initial forms. They found that there is no difference in the permeability of all these types of cells. In order of decreasing speed of penetration into the cells the alcohols form the following series: ethylene glycol, diethylene glycol, triethylene glycol, glycerol. On the basis of these results the authors conclude that the cell membrane has sieve properties.

In LeFevre's book (LeFevre, 1955), the results of a number of workers are given which allow him to speak not of the simple diffusion of glycerol into animal and human erythrocytes but of their transport through the cell envelope (see also Stein, 1962b).

Evidently, the lowered solubility of glycol and glycerol, mannitol and other substances in the protoplasm and their adsorptional binding by the colloids of live tissue play a decisive role in their distribution between cells and medium and also in the distribution of the other substances in experiments which have been described above.

### *5. The Permeability of Cells for Surface-Active Substances*

(monohydric alcohols, aldehydes, ketones, derivatives of monohydric alcohols, etc.)

The penetration into cells of monohydric alcohols, aldehydes, their derivatives and other neutral substances has been studied in detail by many workers, principally in connection with the phenomenon of narcosis (Overton, 1901; Traube, 1910, 1913; Winterstein, 1926; Lazarev, 1938, 1940).

In Overton's series the monohydric alcohols, aldehydes, ketones and ethers are placed among the substances that most quickly penetrate animal and plant cells. They penetrate considerably more quickly than dihydric alcohols and also than urea and its derivatives. Overton (1896, 1902a) reached the above conclusion on the basis of osmotic experiments: in solutions of these substances cells show no narcotic effect. Unfortunately, direct chemical analysis is a technique that has not been used much in the study of the permeability of cells for these substances. But various indirect data (for example, functional and phase changes of the protoplasm under the action of these substances) indicate that all the substances in this group penetrate cells very quickly.

As is well known, Overton (1897, 1902a) connected the easy permeability of cells for these substances with their lipophilia, while Traube (1904a, b, 1908, 1910, 1913) connected it with their surface activity at the water/air boundary.

This easy permeability, as it turned out later, runs parallel not only with the solubility of the above substances in fat-like substances and other organic liquids and with their surface activity at the water/air boundary, but also with their ability to be adsorbed by carbon, denatured proteins and other substances (Fleischman, 1928; Nasonov and Aleksandrov, 1937; Rayevskaya and Troshin, 1937; Utkina, 1938, and others).

Evidently, the adsorption by the protoplasm colloids of these substances plays a leading role in their absorption by cells. In this connection the work of Lazarev and Nusel'man (1932) and Brusilovskaya (1939, 1947) is of great interest: they showed that chloroform, ether and other narcotics are absorbed in very large quantities by the erythrocytes of various mammals.

For example, the chloroform concentration in cells can reach a value many times that of the concentration in the medium. The absorption of chloroform by cells in relation to the concentration in the external liquid obeys, as shown by Lazarev and Nusel'man, Freundlich's formula. However, for some substances in this group (for example, acetone) the coefficient of distribution between erythrocytes and plasma can be less than unity (Lundsgaard and Holböll, 1926; Lazarev, 1938, 1940; Brusilovskaya, 1940, 1947, and others).

Thus, the facts show that in the mechanism of the distribution of this group of substances there is nothing qualitatively new in comparison with the mechanism of the distribution of sugars, creatinine, urea and other substances, whose distribution has been discussed above.

## B. PLANT CELLS AND MICRO-ORGANISMS

### 6. *The Permeability of Plant Cells and Micro-organisms for Non-electrolytes*

The great bulk of the experimental data on the penetration of non-electrolytes into plant cells was obtained by the osmotic method and its several variants. As has been pointed out above, this method does not yield reliable results on the penetration of substances into the protoplasm, though it may be used in certain cases to obtain reliable information about their entry into the central vacuole.

In opposition to Overton, many workers have established by the osmotic method that sugars, though slowly, nonetheless do penetrate the vacuoles of plant cells. It was noticed that disaccharides penetrate more slowly than monosaccharides. This phenomenon was observed by Ruhland (1911), Fitting (1917) and Höfler (1918, 1926) in the cells of beet, spiderwort and other plants.

Urea, according to all authors, penetrates plant cells very quickly, but more slowly than monohydric alcohols (de Vries, 1889; Fitting, 1919; Höfler and Stiegler, 1921; Ruhland and Hoffmann, 1925, and others).

Collander and Bärlund (1933), using chemical methods, obtained interesting data on the permeability of the cells of the algae *Characeae*. Part of their results are shown in Table 27.

These writers concluded that the penetration of substances into the vacuole depends on their ability to pass through the protoplasmic membrane, whence it follows that sugars and monohydric alcohols either do not penetrate the cell at all, or only extremely slowly.

Höfler (1934) obtained similar results by the plasmometric method in the case of *Majanthemum bifolium* cells, but reached completely different conclusions. In opposition to Collander and Bärlund and many other writers, he asserts that all the substances investigated by him pass into the protoplasm without hindrance, but the vacuole envelope—tonoplast—is penetrable only with great difficulty for some substances.

TABLE 27. THE TIME OF PENETRATION OF NON-ELECTROLYTES INTO THE CELL SAP  
OF *Chara ceratophylla*. THE TIME INDICATED IS THAT NEEDED TO REACH HALF  
THE EXTERNAL CONCENTRATION  
(from Collander and Bärlund, 1933)

Substance	Min	Substance	Min
Methyl alcohol	1.3	Dimethylurea	38
Ethyl alcohol	2.3	Ethyurea	100
Urethane	3.0	Thiourea	170
Triethyl citrate	3.5	Methylurea	190
Trimethyl citrate	5.5	Urea	320
Cyanamide	6.1	Glycerol	1700
Propylene glycol	15	Erythritol	28000
Acetamide	24	Arabinose	42000
Ethylene glycol	30	Glucose	42000
		Sucrose	42000

Höfler's data are given in Table 28, in which, for comparison, the results of Collander and Bärlund are also given. This table shows the relative speeds of penetration of substances into plant cells.

TABLE 28. THE PERMEABILITY OF *Majanthemum bifolium* AND *Chara ceratophylla* CELLS  
TO NON-ELECTROLYTES. THE PERMEABILITY TO GLYCERIN IS TAKEN AS UNITY  
(from Höfler, 1934)

Substance	Majanthemum	Chara	Substance	Majanthemum	Chara
Water	120-180	13300	Glycerol	1	1
Urea	2.76	5.4	Erythritol	0.076	0.063
Methylurea	7.96	9.2	Glucose	0.068	0.042
Thiourea	9.43	10.2	Fructose	0.091	0.042
Lactamide	3.44	7.5	Sucrose	0.030	0.042
Malonamide	0.56	0.192	Maltose	0.091	0.042

Interesting information on the penetration of a number of non-electrolytes into the cells of the dead nettle (*Lamium album*) is given by Schmidt (1939). Using the plasmometric method suggested by Höfler (1918), he determined the "permeability constant *P*" for the cells of plants grown under different moisture conditions and described by him as "hydrated" and "dry". His results are given in Table 29.

The values obtained show that the permeability coefficient of the "dry" cells is considerably higher for some substances, and considerably lower for others, than that of the "hydrated" cells. These results could be explained by a change in the properties of the semi-permeable membrane such that the membrane becomes more dense in the "dry" cells, as a result of which the

permeability coefficient for urea and its derivatives is less than for "hydrated" cells. However, it then becomes impossible to understand how the permeability of these cells for fructose is increased.

TABLE 29. THE PERMEABILITY OF THE EPIDERMAL CELLS OF *L. muculatum*  
FOR NON-ELECTROLYTES  
(from Schmidt, 1939)

"Hydrated" plants	<i>P</i>	"Dry" plants	<i>P</i>
Thiourea	3.37	Ethylene glycol	7.26
Ethylene glycol	1.73	Glycerol	3.38
Urea	1.54	Urea	1.04
Glycerol	1.32	Lactamide	0.991
Lactamide	1.015	Methylurea	0.839
Methylurea	0.915	Thiourea	0.333
Malonamide	0.175	Malonamide	0.125
Erythritol	0.00536	Erythritol	0.0219
Fructose	0.00109	Fructose	0.00125

As has been mentioned, some writers consider that cells are completely impermeable for sugars. It is difficult to agree with such an assertion in view of the major role played by these substances in the metabolism of any cell. It is possible that carbohydrates find difficulty in passing through the tonoplast into the central vacuole, but they should penetrate the mesoplasm freely. This is confirmed by the observations of many writers (Puriyevich, 1898; Wächter, 1905; Krassinskii, 1930; Mason, Maskell and Phllis, 1936, and others).

Very interesting and important is the work of Kursanov (1936), Sisakyan (1936, 1937), Kursanov and Kryukova (1937a, b), Lisitsin (1937), Shcherbakov (1938) and others, who showed that on the vacuum-infiltration of sugars there is observed in the leaves of plants an increase in the fermenting action of invertase, both the rate of synthesis of monosaccharides on infiltration and the speed of hydrolysis of saccharose being very high, which indicates that large amounts of the infiltrated sugars pass into the cell. Thus, according to the calculations of Kursanov and Kryukova (1937a), sucrose, after infiltration into live cyclamen leaves (100–125 mg per 1 gm dry weight of leaves) passes out of the extracellular spaces into the cells at a rate of 27.6–28.3 mg per hr per 1 g dry weight of tissue.

A fact worthy of notice is that, according to the results of many workers, sugars can move into plant cells "against the concentration gradient" and accumulate there in large quantities (Phllis and Mason, 1933, 1936; Lundgårdh and Burström, 1944–1945).

Sabinin (1940) reviews many reported results which indicate that sugars diffuse into the protoplasm of cells (from cell to cell along the phloem) at

a speed greater than their speed of movement in free diffusion. In this connection very convincing results are given by Mason and Maskell (1928), Mason and Phillis (1936a), Phillis and Mason (1936), experimenting with the cotton plant, and also by Crafts (1932), who observed the same phenomenon in experiments with cucumbers. More recently, convincing results which point in the same direction have been obtained by Kursanov and Zeprometov (1949), Kursanov (1952) and Kursanov and Turkina (1952).

Horie (1954), using the osmotic method, studied the permeability of cells of the algae *Hydrodictyon reticulatum* to a number of organic substances. According to this writer, sucrose permeates into cells slowly. For a number of substances the permeability of the cells is higher, the lower the molecular weight and the higher the surface activity of the substance.

The permeability of yeast to non-electrolytes has been the subject of much work, done principally by osmotic methods. The results so obtained are conspicuous for their extreme inconsistency. Swellengrebel (1905) found by the plasmolytic method that sugars pass into yeast cells very slowly. It is interesting that urea, according to his observations, also penetrates these cells very slowly. Ethyl alcohol was found to penetrate very quickly, mannitol very slowly, glycerol occupying an intermediate position in this respect. According to Swellengrebel, chloral hydrate apparently does not penetrate yeast at all nor has any narcotic effect, whereas ethyl ether penetrates instantaneously and causes narcosis of the cells. In his opinion, the penetration of substances into cells depends on the presence in the envelope of relevant lipoids, in which the substance penetrating is to dissolve. If there are no such lipoids in the envelope, then the substance cannot penetrate it.

Paine (1911) attempted to determine gravimetrically the permeability of yeast to water, alcohol, acetone, glycerol, urea and a number of mineral salts. The yeast cells were weighed twice: before their immersion in the solutions of these substances and at a fixed time after immersion. The writer was unable to obtain any definite results by this method. Chemical analysis permitted Paine to discover that alcohol penetrates yeast cells very quickly. At an alcohol concentration in the medium of from 5 to 20 per cent diffusion equilibrium is reached in 3 hr, the equilibrium concentration in the cells reaching 85 per cent of the medium, which indicates, in the opinion of the writer, that there is a certain amount of bound water in the cells.

The same method with account taken of the change of water in the cells was used by Söhngen and Wieringa (1926) to determine the penetration into yeast of lactose, sodium chloride and urea. They established that urea passes into these cells 13 times more quickly than sodium chloride.

Rubinshtein and Verkhovskaya (1935) studied the change in volume of yeast in solutions of sodium chloride, glycerol and urea, prepared in Fisher's nutritive medium. The change in volume of the cells was measured by centrifugation in special tubes. The authors found that plasmolysed cells (diminished in volume) do not regain their former volume with the passage of time.

They connect this phenomenon with the special properties of the yeast envelope, which, so they suppose, is conspicuous for its extremely low elasticity. In view of this, none of the experimental results on the permeability of yeast obtained by osmotic methods can be considered reliable.

The change in volume of brewer's yeast in solutions of lactose and sodium chloride has been studied by the present writer (Troshin, 1948a). It was found that as the concentration of these substances in the medium increased, the volume of the cells decreased, but not according to the law of osmosis. The reduction in volume occurs simultaneously with the penetration of lactose and chloride ions into the cells, which prevents our regarding the dehydration of the cells as an osmotic phenomenon.

In spite of the assertions of many writers that sugars pass very slowly into cells, Slator and Sand (1910) showed that the speed of diffusion of these substances through yeast envelopes is considerably greater than their speed of diffusion in pure aqueous solution. The unusually high permeability of yeast to glucose is also noticed by Ørskov (1945).

Further, Wertheimer (1934) observed very strong adsorption of sucrose by yeast cells. His results are analogous to those of Lundegårdh and Bürstrom (1944–1945) in their study of the distribution of glucose and galactose between the cells of wheat roots and the medium. The latter, as has already been noted, discovered the passage of carbohydrates at a low concentration into the cells "against the concentration gradient".

To elucidate the significance of the sorptional factors (solubility, chemical and adsorptional binding) in the process of the absorption of sugars by plant cells I studied the distribution of lactose between the yeast cells *Saccharomyces ellipsoideus* and a medium (Troshin, 1951c). Lactose, as is well known, is not fermented by these cells.

For our purposes it was important that at the end of the experiment, at all the lactose concentrations in the medium tried, the ratio of dry residue to water in the yeast remain constant because, by analogy with coacervates it could be expected that the dehydration of the cells would result in lowered solubility of the sugar in the protoplasm. The requisite constancy of water content was achieved by a corresponding reduction in the amount of sodium chloride in the physiological solution in which the sugar solutions were prepared. The concentration of lactose in the yeast was calculated from the diminution of the lactose concentration in the surrounding medium.

First of all the passage of lactose into yeast cells with time was studied. The results of this series of experiments are given in Fig. 34, from which it can be seen that diffusion equilibrium between the sugar in the medium and in the yeast was reached in 3 hr. Water equilibrium is established in the same period. Thus, if the concentration of lactose ( $C_s$ ) was 18·9 per cent at the beginning of the experiment, then it fell sharply, reaching 10·53 per cent in 3 hr and then not changing substantially thereafter (curve A in Fig. 54a). Correspondingly, there is a rapid growth in the sugar concentration in the

yeast: it reaches 8·12 per cent in 3 hr and then also does not change (curve B in Fig. 54a).

Equilibrium is reached just as quickly at lower initial concentrations of lactose in the medium (3·1 and 2·3 per cent); but the ratio of the sugar concentrations in the cells and the equilibrated liquid is completely different. If in the first case (Fig. 54a) the sugar concentration in the yeast at equilibrium is

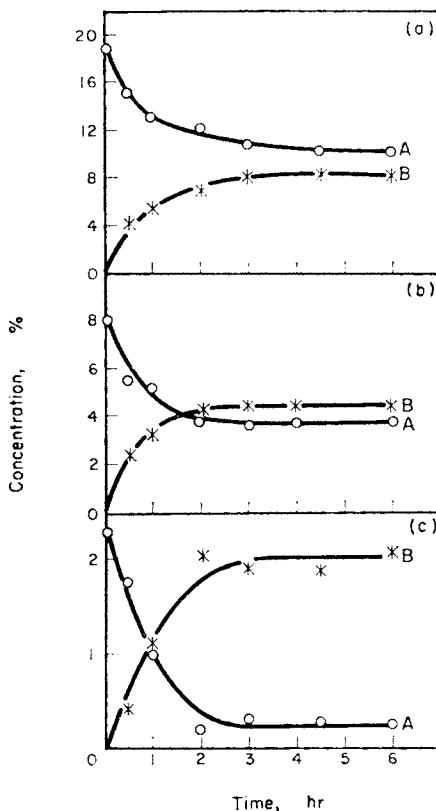


FIG. 54. Curves of the absorption of lactose by yeast with time.

Abscissae—the length of the experiment, ordinates—the lactose concentration in the medium (curves A) and in the yeast (curves B). Initial lactose concentration in the medium: a—18·9 per cent, b—3·1 per cent, c—2·3 per cent.

23 per cent lower than in the medium, then in the second case (Fig. 54b) it is 9 per cent, and in the third case (Fig. 54c), it is 447 per cent greater than in the surrounding liquid.

To study this dependence in more detail another series of experiments was carried out with intermediate initial lactose concentrations, the duration still being 3 hr. The results are shown in Table 30.

TABLE 30. THE AMOUNT OF LACTOSE IN YEAST CELLS AT VARIOUS CONCENTRATIONS  
OF LACTOSE IN THE EQUILIBRATED LIQUID  
(in g per 100 ml of water)

Expt. No.	Lactose concentration		Water content of the yeast (in %)	Of the total lactose in the yeast cells		$Q = \frac{C_c}{C_s}$
	in the medium $C_s$	in the yeast $C_c$		dissolved part $C = C_s K$	adsorbed part $A = C_c - C$	
1	2	3	4	5	6	7
1	0.13	0.93	68	0.07	0.86	7.16
2	0.32	1.75	68	0.18	1.57	5.47
3	1.16	2.84	69	0.64	2.20	2.45
4	3.92	4.28	69	2.17	2.11	1.09
5	5.54	5.25	70	3.05	2.20	0.95
6	10.53	8.12	67	5.79	2.33	0.77
7	15.40	9.21	58	—	—	0.51

In the fourth column of this table we give information about the water content in the yeast at various equilibrium concentrations of lactose in the surrounding liquid. As can be seen, even with very wide variation in the sugar concentration in the medium the percentage of water in the yeast remains almost constant, except for the last group of experiments, where the equilibrium concentration of sugar in the medium was very high (15.4 per cent).

The seventh column contains the ratios ( $Q$ ) of the sugar concentrations in

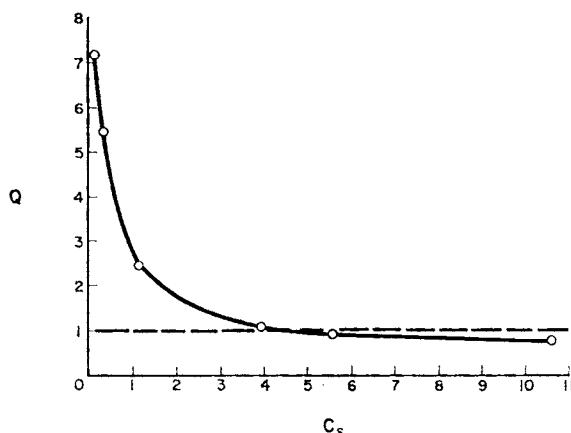


FIG. 55. The dependence of the coefficient  $Q$  on the lactose concentration in the surrounding equilibrated liquid ( $C_s$ , in per cent) when lactose is distributed between yeast and the surrounding medium.

the yeast ( $C_c$ ) to the concentrations in the medium ( $C_s$ ). These ratios show how much more or less lactose there is in the yeast in comparison with the amount in the medium. Thus, when  $C_s = 0\cdot13$  per cent,  $Q = 7\cdot15$ , i.e., there is approximately seven times as much lactose in the yeast as in the medium, while, when  $C_s = 15\cdot4$  per cent,  $Q = 0\cdot51$ , i.e., there is approximately half as much lactose in the yeast. This dependence of  $Q$  on  $C_s$  is

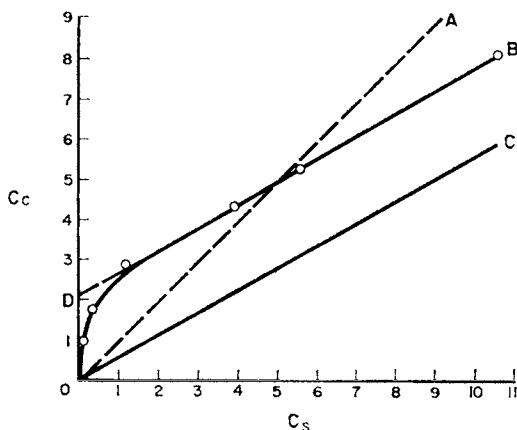


FIG. 56. The dependence of the lactose concentration in yeast ( $C_c$ , in g per 100 ml of intracellular water) on its concentration in the medium ( $C_s$ , in per cent).

portrayed in Fig. 55. This curve is analogous to those which characterise the distribution of galactose and sucrose between a coacervate and its balanced liquid and also that of many non-electrolytes between the muscles, blood elements and medium. This coincidence in the curves of the dependence of  $Q$  on  $C_s$  is not fortuitous, but the result of common causes in all these cases, namely, that the sugar content of yeast cells depends on the ability of the sugar to be adsorbed by the protoplasm colloids and on the solubility of the sugar in the latter.

The results obtained permit us to determine graphically to a sufficient degree of accuracy, as was done in the experiments with coacervates, erythrocytes and muscles, what part in the distribution of sugar is to be attributed to adsorption and what to solution in the protoplasm. In Fig. 56 we show the dependence of the lactose concentration in the yeast on the concentration in the surrounding equilibrated liquid. If the sugar which penetrates the yeast were as soluble there as in the water of the surrounding medium and were not bound by the cell colloids, then we should find the non-electrolyte concentrations inside and outside the cell to be equal: this would be represented by the line OA in Fig. 56. However, the dependence of the sugar concentration in the yeast on the concentration in the medium is different

in character. It corresponds to the curve OB and obeys the law:  $C_c = C_s K \{1 + A_\infty / (C_s K + a)\}$ .

As there is no substantial change in the ratio of the dry residue to the water in the yeast within the limits of the sugar concentrations in the medium shown in Fig. 56, it is easy to determine the constants  $A_\infty$  and  $K$  in the formula from this graph. The sector OD of the ordinate axis is numerically equal to the limit of adsorption of lactose by yeast ( $A_\infty$ ). In this case  $A_\infty = 2.2$  g of lactose per 48–49 g of dry residue of yeast, which corresponds to 100 ml of water in the cells (see Table 30). If we now draw a line OC from the origin of coordinates parallel to DB, we thus divide the sugar dissolved in the protoplasm from that adsorbed in it. The line OC will reflect the dependence of the concentration of dissolved sugar in the cell on the concentration in the balanced liquid. In the present case we obtain a relation obeying the law  $C = C_s K$ , where  $K = 0.55$ , that is, there is dissolved in the water of the yeast protoplasm, within wide limits of variation of the lactose concentration in the equilibrated liquid, 45 per cent less of this non-electrolyte than there is in the water of the surrounding medium.

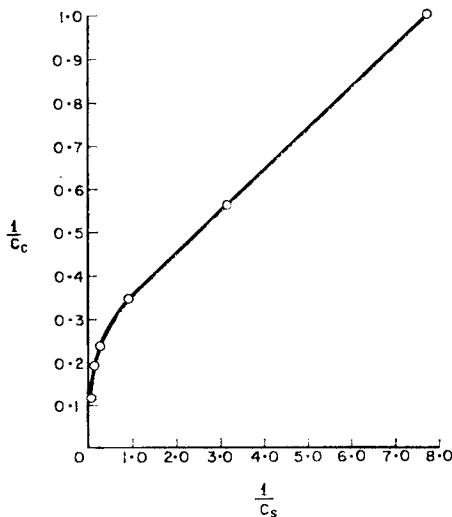


FIG. 57. The dependence of the reciprocal of the lactose concentration in yeast  $1/C_c$  on the reciprocal of the concentration in the medium  $1/C_s$ .

If the lactose distribution obeyed only Henry's law, then we should have a linear dependence of the lactose concentration in the yeast ( $C_c$ ) on its concentration in the medium ( $C_s$ ). In which case the equation  $C_c = C_s K$  would apply, which does not in fact happen.

If the distribution of lactose between the yeast and the medium were a purely adsorptive process, then the curve OB in Fig. 56 would obey Lang-

muir's equation or Freundlich's empirical formula. This assumption can be tested by writing Langmuir's and Freundlich's equations in linear form:

$$\frac{1}{C_c} = \frac{1}{A_\infty} + \frac{a}{A_\infty} \times \frac{1}{C_s} \text{ (Langmuir's equation);}$$

$$\log C_c = \log a + n \log C_s \text{ (Freundlich's equation).}$$

As can be seen from Figs. 57 and 58, a linear relation between  $1/C_c$  and  $1/C_s$  and  $\log C_c$  and  $\log C_s$  is not to be seen.

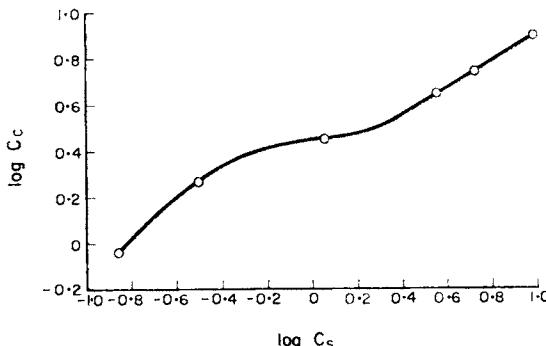


FIG. 58. The logarithmic dependence of the lactose concentration in yeast ( $\log C_c$ ) on its concentration in the medium ( $\log C_s$ ).

Thus we conclude that the distribution of lactose between yeast and the medium, just like the distribution of non-electrolytes between animal cells and medium and between a coacervate and the equilibrated surrounding liquid, includes both distribution proper (according to Henry's law) and adsorption (or chemical binding).

Table 30 contains experimental data and also the concentrations of dissolved and adsorbed sugar as the difference between the total sugar content of the cells as found experimentally and the dissolved part calculated from the formula:  $C = C_s K$ , where  $K = 0.55$ .

Of course, we cannot claim absolute accuracy for these data, but all the same they give a good picture of the parts played by adsorption and solution respectively in the distribution of lactose between live yeast cells and the medium surrounding them. They also make it clear why, at low concentration in the medium, there is more sugar in the cells than in the surrounding medium, and why, at high concentrations of lactose, the reverse relation holds.

For an eightyfold change in the lactose concentration in the medium there is altogether a ninefold change in the yeast, the quantity  $Q$  falling rapidly with increasing sugar concentration from 7.16 to 0.77. It can be seen from Table 30 that this phenomenon is due to the fact that, for all the concen-

trations shown, there is 45 per cent less dissolved sugar in the protoplasm water than in the medium, and the concentration in the cells is reduced in proportion to the reduction in the lactose concentration in the medium. However, the amount of lactose adsorbed by the yeast falls less than three times while the amount in the medium is reduced eightyfold. If there is a 10·53 per cent solution of lactose in the medium, there is about half as much adsorbed sugar in the cell than there is dissolved, whereas, if the solution in the medium is 0·13 per cent lactose, there is now 12 times more adsorbed sugar than dissolved. This is due on the one hand to the relatively small change in the sugar concentration in the cells for very large changes in its concentration in the surrounding equilibrated liquid and, on the other, to the prevalence of sugar in the yeast at low concentrations and its prevalence in the medium at high concentrations.

The data in Table 30 allow us to determine the constant  $\alpha$  in the equation given above. In Fig. 59 we have the lactose adsorption isotherm with the adsorption limit  $A_\infty$  indicated. The constant  $\alpha$  is equal to 0·07. The circles in the figure denote the mean values from Table 30 and the crosses the values obtained by calculation from Langmuir's formula with  $A_\infty = 2\cdot2$  and  $\alpha = 0\cdot07$ . As can be seen, the coincidence between the two is almost complete. This is satisfying proof of the applicability of the formula to the distribution of sugar between cells and medium.

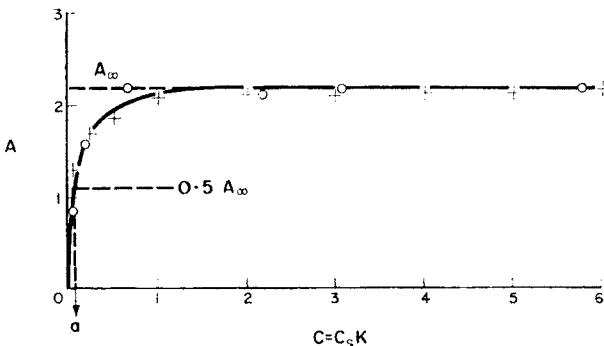


FIG. 59. The isotherm of the adsorption of lactose by yeast cells.

Abcissae—the concentrations of the dissolved lactose in the water of the yeast (in per cent), ordinates—the amount of adsorbed lactose (in g per 100 ml of intracellular water or per 48–49 g of dry residue of yeast).

On the basis of the above, we may conclude that yeast cells, like animal cells, have a mechanism which ensures a more or less constant level of sugar in them in spite of considerable variations in the concentration in the medium. The principal physico-chemical factors in this mechanism are the lowered solubility of the non-electrolyte in the protoplasm and its adsorption by the colloids of the living substance. At large sugar concentrations in the me-

dium its concentration in the cells is due principally to the former factor, its lowered solubility, while at low concentrations, adsorption comes to the fore, ensuring a considerable excess of the non-electrolyte in the cell over its concentration in the surrounding medium.

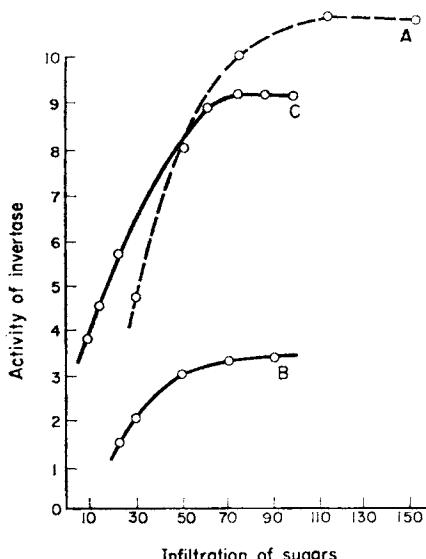


FIG. 60. The dependence of the speed of hydrolysis or synthesis (in mg of invert sugar) on the concentration of infiltrated sugars (in mg per g of dry weight of leaf) in the leaves of cyclamen and chicory (from Kursanov, 1936).

A—cyclamen, hydrolysis; B—cyclamen, synthesis; C—chicory, synthesis.

These physico-chemical factors, in my opinion, should play an important biological role. Evidently, it is thanks to them that a uniform (and sufficient) flow of nutriment passes into the cells while there are wide variations in the concentration in the medium, and that thus the succession of biochemical reactions at a definite level is ensured.

In support of this proposition we have numerous results which give information about the properties of enzymes. Thus, it is well known that the activity of enzymes has a definite relation to the concentration of substrate. The speed of an enzymatic process increases quickly with increasing concentration of substrate, reaching a definite level and then remaining unchanged in spite of large increases in its concentration in the medium (Michaelis-Menten rule). The curve of the dependence of the speed of an enzymatic process on the concentration of substrate looks like an adsorption isotherm. The curves in Fig. 60 show this pictorially.

Many writers suppose that this type of curve for the speed of an enzymatic reaction is connected with the fact that there is at its basis heterogenous catalysis requiring contact of the enzyme with the substrate. It is interesting

that the isotherm obtained for the adsorption of lactose by yeast exactly coincides with the curve of the speed of fermentation of glucose obtained by Slator (1906) and with the curves of the activity of invertase given by Hitchcock and Dougan (1935), Afanas'yev and Il'yina (1949), Il'yina (1950) and other workers. Data of a similar kind are also to be found in Hinshelwood's book (1946).

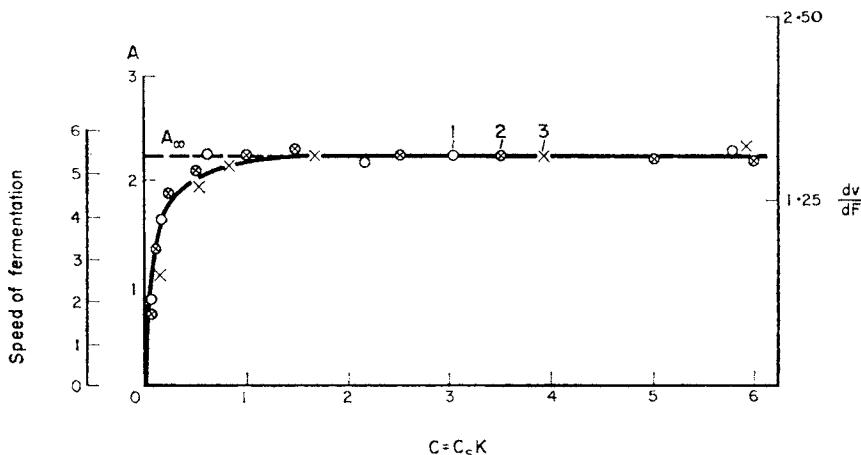


FIG. 61. The isotherm of the adsorption of lactose by yeast and the dependence of the activity of the enzymes on the concentration of substrate in the medium.

Abscissae—the lactose concentrations in the water of the yeast (or the corresponding concentrations of sugars in the medium), ordinates—the amount of lactose adsorbed by the yeast ( $A$ , in g/48–49 g of dry weight of yeast), the activity of the invertase ( $dV/dF$  after Afanas'yev and Il'yina, 1949) and the speed of fermentation of glucose (in ml, from Slator, 1906).

1—the adsorption of lactose, 2—the activity of the invertase, 3—the speed of fermentation of the glucose.

In Fig. 61 it can be seen that the coincidence of the curves of the speeds of enzymatic reactions with the isotherm of lactose adsorption is complete: the bends in the adsorption curve and the curve of the speed of enzymatic reaction occur at identical sugar concentrations. It is my opinion that this coincidence is not accidental. If it were at the basis of enzymatic reactions of heterogenic catalysis and if the speed of any part of the enzymatic transformation of a substance were less than the speed of entry of sugar into the cell, then we should inevitably find that the adsorption isotherm and the curve of the speed of the enzymatic reaction were one and the same in form.

In the analysis of the experimental data we took into account only those obtained at a more or less constant water content of the yeast. As follows from Table 30, if the amount of water in the cells is sharply reduced (at a lactose concentration in the medium of 15·4 per cent), the percentage absorption of lactose by the yeast falls considerably compared with that at lower

lactose concentrations in the medium. Thus, when  $C_s = 15.4$  per cent, the lactose content in the yeast  $C_c = 9.21$  per cent, whereas for the given values of  $A_\infty$ ,  $K$  and  $a$  we should have  $C_c = 10.8$  per cent. This lowering of the sugar content is probably due to the fact that in the present case the water content in the cells is about 10 per cent lower than normal, as a result of which the solubility of the sugar in the protoplasm water is reduced. This phenomenon corresponds to that concept of the state of the water in the protoplasm, according to which there is no free water in the cells, but that it is all bound as it is in coacervates.

Water emerges from the cell in the first place because the molecules are less firmly bound, but as a result the activity of the remaining water as a solvent is still further lowered.

To the present time the concrete mechanism of the penetration of carbohydrates into the cells of microorganisms remains unexplained. Many research workers assert that all monosaccharides and disaccharides do not pass into yeast cells independently of whether the sugars are fermented by such cells or not. Thus, Conway and Dawney (1950) suppose that arabinose and galactose do not permeate into the cells of baker's yeast. A number of authors prove that the permeability of bacterial cells to sugars and other metabolites is controlled by specific transport systems ("permeases") of an enzymatic character. They assert that for every metabolite penetrating a cell there must be the corresponding permease (Cohen and Mound, 1957; Kepes, 1958; Horecker, Osborn, McLellan, Avigad and Asensio, 1961; Sols and de la Fuente, 1961). Rothstein (1954a, b, c, 1956) showed that sugar molecules as such (in their unchanged form) do not penetrate yeast cells; in his opinion, the sugars utilised by cells are subjected to phosphorylation in the cell membrane and thus pass into the cell in the form of phosphoric esters. However, such ideas were not confirmed by the later work of other writers (Sols, 1946; Burger, Hejmova and Kleinzeller, 1959; Cirillo, 1961; Kotyk, 1961, and others).

Burger *et al.* (1959) found that on the incubation of yeast in a 5 per cent solution of sugars not utilised by these cells with a phosphate buffer at 30° only the monosaccharides pass into the yeast cells, while the others do not. Thus, *d*-arabinose and *d*-galactose pass at great speed into the cells *Saccharomyces cerevisiae*, while *d*-galactose also passes into the cells of *S. fragilis*; *d*-methylglucoside passes into the cells of *S. fragilis* but not into *S. cerevisiae*. According to these authors, maltose and lactose do not penetrate the yeast cells of *S. cerevisiae* and *S. fragilis*, while trehalose passes into cells of the first kind, but not into cells of the second. According to the experiments of Cirillo (1961), in the same conditions the cells of baker's yeast are freely permeable for sugars not fermented by these cells, such as *l*-sorbose, *d*-xylose and *d*-methylglucoside, while *d*-galactose, *l*-arabinose,  $\alpha$ -*d*-methylmannoside and lactose do not penetrate these cells.

According to the calculations of Burger and his colleagues and Cirillo,

all the water of yeast cells is available for the solution of the sugars penetrating the cell, so that at the moment of equilibrium (after 40–60 min) the concentration of sugar in the water of the cell becomes equal to the external concentration. However, the arguments brought forward in support of this conclusion are not at all convincing. In the work of Burger *et al.*, the yeast samples were washed three times in cold salt solution before analysis (after their incubation in the sugar solution). This treatment would cause a considerable amount of the sugar to come out of the cells into the solution, as was shown in the experiments of Cirillo (1961). Consequently, the sugar concentration in the cells was in fact higher than in the medium. Another pointer in this direction is the adsorptional character of the dependence of the concentration of *L*-arabinose in the cells of *S. cerevisiae* on the concentration of this sugar in the medium (Burger *et al.*, 1959, p. 235, Fig. 2). In Cirillo's calculations of the sugar concentration in the cells it was assumed that 30–33 per cent of the water of the cell residue was to be attributed to extracellular water. This is too high a percentage for the water of the extracellular spaces. These methodological factors also evidently explain why the authors of these two works were driven to the conclusion that a number of sugars entirely fail to penetrate yeast cells.

Burger and his colleagues found that the entry of galactose into cells and its exit from them are characterised by very high values of the coefficient  $Q_{10}$  (2·9 and 2·4 respectively). The maximal (initial) speed of entry of galactose and arabinose into the cells of *S. cerevisiae* is equal to 2·06 µg/mg of dry weight of cells/min. The entry of galactose into these cells is very strongly depressed by glucose, glucosamine hydrochloride and iodoacetate, while glucose-1-phosphate, glucose-6-phosphate, maltose, trehalose and  $\alpha$ -*d*-methylglucoside have no effect on the speed of penetration of galactose into the cells. It is interesting that glucose, when added to a suspension of yeast that has previously been incubated in a galactose solution, forces the galactose out of the cells; in this case the emergence of the galactose from the cells into the medium works against the concentration gradient.

On the basis of the above data, Burger and his colleagues suppose that sugars pass into the cells or emerge from them by means of membrane carriers ("carrier-linked"). Cirillo differentiates two phases in the penetration of sorbose into yeast cells: a fast penetration phase which does not require the expenditure of energy—"activated diffusion", the second phase being slower and requiring the expenditure of metabolic energy—"active transport".

However, the above facts do not contradict the sorptional theory of permeability. Competitive relations in the process of the passage of sugars into cells, their accumulation in the protoplasm and the expulsion from cells of one sugar by another can be explained by adsorptional-desorptional phenomena and the poor solubility of sugars in the water of the protoplasm as a whole as well as by processes that take place on the surface of cells, in a membrane.

The data given in this section of the chapter, both from the literature and from the writer's own work, indicate that the distribution of non-electrolytes between plant cells and medium is regulated by the same factors as regulate their distribution in the systems: animal cells/medium and coacervate/equilibrating liquid. These factors are the lowered solubility of non-electrolytes in the protoplasm and the ability of these substances to be adsorptionally or chemically bound by the cell colloids. The binding of non-electrolytes by the colloids and the reduced solvent power of the protoplasm ensure the preponderance of the concentration of the penetrating substance in the cell over its concentration in the medium at low concentrations and the reverse relation at higher concentrations.

### *7. Conclusions*

1. Notwithstanding the conclusions of the classical membrane theory, carbohydrates (monosaccharides and disaccharides), like urea, creatinine, surface active substances and other non-electrolytes penetrate into a wide variety of animal and plant cells.
2. In conditions of diffusion equilibrium the concentration of many non-electrolytes in the cell at low concentrations is higher than in the medium, while at higher concentrations the reverse obtains. At low concentrations of many non-electrolytes in the medium their diffusion into the cell takes place "against the concentration gradient".
3. The phenomenon described in the preceding paragraphs is due not to the properties of the membrane, but to the sorptional properties of the protoplasm.

A substance penetrating a cell can be in either the dissolved or the bound state. The solubility of non-electrolytes in the protoplasm is 2–3 times less than in the aqueous surrounding medium, the concentration of the dissolved fraction in the cell increasing in direct proportion to the increase in the medium.

The simultaneous action of the sorption factors (solubility, adsorption and chemical interaction) ensures the preponderance of the non-electrolyte concentration in the cell at low concentrations and, on the other hand, the preponderance of the concentration in the medium at higher concentrations.

4. The biological significance of this phenomenon is, evidently, that, thanks to this mechanism of the distribution of substances, a cell can absorb from the medium a sufficient amount of nutriment from very dilute solutions and also protect itself from excess penetration from strong solutions.

## CHAPTER VI

# The Permeability of Cells for Certain Organic Acids (Amino Acids, Ascorbic Acid, etc.)

### 1. *The Permeability of Cells for Amino Acids*

*Formed elements of the blood.* The study of the permeability of cells for amino acids has always attracted much attention from physiologists and biochemists because of the important part played in the life of cells by these compounds which go into the synthesis and the renewal of the protoplasmic proteins.

Many investigators who have studied the permeability of erythrocytes by the osmotic technique have asserted that these cells are completely impermeable for amino acids (Grijns, 1896; Kozawa, 1914; Hiruma, 1923, etc.). In contradiction to these assertions, direct chemical analysis and the radioactive techniques have established that animal and human erythrocytes absorb very quickly and in large quantities a great variety of amino acids, a process which, as has been noted by many writers, is reversible.

Here we should first of all introduce certain data relating to the distribution of amino acids between the formed elements and the plasma of human blood and that of various animals. Part of this material from the literature is shown in Table 31, where we give the mean values of the amino nitrogen or the extreme variations of these quantities.

Hamilton and Van Slyke (1943), using the ninhydrin gasometric method, which permits the determination of the nitrogen of only those amino groups which are in the  $\alpha$ -position to free carboxyl groups, showed that the  $\alpha$ -amino nitrogen content of erythrocytes is normally greater than that of the plasma. Thus, analysing the blood of a group of donors, they found that the concentration of  $\alpha$ -amino nitrogen in the plasma was 4.07 mg per cent, while in the erythrocytes it was 7.65 mg per cent: the amount of total amino nitrogen found by normal methods was, respectively, 4.36 and 10.88 mg per cent. Similar results were obtained by Christiansen and Lynch (1946), who also determined the distribution of  $\alpha$ -amino nitrogen.

In rabbit reticulocytes the glycine concentration is 3–5 times greater than in the blood plasma. If glycine is added to the medium, it passes into these cells “against the concentration gradient”. High concentrations of cyanide, arsenate and dinitrophenol depress the accumulation of glycine in reticulo-

TABLE 31. THE NORMAL DISTRIBUTION OF AMINO ACIDS IN THE BLOOD OF HUMANS AND VARIOUS TYPES OF ANIMALS

Subject	Number of determinations	Amount of amino nitrogen (mg%)			$Q = \frac{C_c}{C_s}$	Author
		whole blood	erythrocytes ( $C_c$ )	plasma ( $C_s$ )		
1	2	3	4	5	6	7
Horses						
full-grown	11	14.54	29.39	6.55	4.49	Saichuk (1941)
foals	12	14.16	29.08	6.66	4.46	Saichuk (1941)
	18	14.29	29.09	6.32	4.60	Saichuk (1941)
Dogs						
	15	11.2-14.6	14.9-19.1	8.2-12.0	1.5-2.1	Kulakova (1939)
	5	10.79	16.92	6.70	2.5	Zbarskii (1936)
	2	8.97-9.15	10.22-10.47	8.01-8.40	1.21-1.30	Fridlyand (1939)
	6	10.2-11.9	13.2-18.6	7.4-9.8	1.49-2.36	Demin (1941a, b)
	6	10.2-12.4	11.2-15.3	8.8-11.1	1.20-1.40	Demin (1941a, b)
Rabbits						
	5	8.43	12.90	6.32	2.04	Barkhash (1934)
	14	10.55	15.43	8.12	1.84	Kheifets (cit. by Zbarskii and Demin, 1949)
	38	13.60	20.85	9.98	2.08	Gur'yeva (1940)
Guinea pigs						
	5	9.5-12.72	10.61-18.16	6.85-10.00	1.27-2.65	Fridlyand (1939)
	5	7.29-8.29	10.73-13.25	5.27-5.46	2.02-2.43	Fridlyand (1939)
Humans						
	20	7.43	9.47	5.52	1.71	Wu (1922)
	12	6.4	8.2	5.3	1.55	Folin and Berglund (1922)
	14	8.32	11.37	6.20	1.83	Gur'yeva (1940)
	5	8.33	10.37	6.81	1.52	Yampol'skaya (1938)
	17	6.44	8.82	4.85	1.82	Sveshnikova (1943)
	400	6.30	—	—	—	Green, Sandiford and Ross (1923-4)

cytes, a phenomenon which is not observed in experiments with the mature erythrocytes of various animals (Riggs, Christensen and Palařine, 1952).

Other formed elements of the blood, in particular leucocytes, have, evidently, an even greater capacity to absorb amino acids than erythrocytes, as can well be seen from the results of Okada and Hayashi (1922), which are shown in Table 32.

TABLE 32. THE DISTRIBUTION OF AMINO NITROGEN BETWEEN THE LEUCOCYTES AND BLOOD PLASMA OF LEUKEMIC DOGS (from Okada and Hayashi, 1922)

Expt. No.	Amount of amino nitrogen (mg%)			$Q = \frac{C_c}{C_s}$
	whole blood	white blood cells ( $C_c$ )	plasma ( $C_s$ )	
51	17.81	32.88	5.18	6.33
52	11.77	37.57	4.80	7.83

Thus, the work of the above mentioned writers has established the strong preponderance of the amino acid concentration in erythrocytes over their concentration in plasma. This preponderance will be even more pronounced if the amount of amino nitrogen in the erythrocytes and the plasma is recalculated in relation to the water content, because the water in the formed elements amounts to about 65 per cent, while it is 90 per cent in the plasma (Ponder, 1934).

The material set out in Tables 31 and 32 and other data show that the formed elements of the blood of animals and humans have considerable sorptional capacity in respect to amino acids.

Further, Folin and Denis (1912) established that shortly after its introduction into the bloodstream of an animal alanine is no longer to be detected in the blood plasma. Hence these workers concluded that alanine is very quickly absorbed from the blood by the cells of various tissues and organs. Also Abderhalden and Kürten (1921) found that peptone, polypeptides and amino acids are absorbed in large amounts by blood cells. In their opinion, these substances are adsorbed on the surface of erythrocytes, the process obeying Freundlich's formula.

Later work by Zbarskii (1925a, b) showed that on the introduction of amino acids into the blood of an animal, they are absorbed preferentially by the erythrocytes. Zbarskii (1925a) introduced into rabbits parenterally the product of prolonged hydrolysis of meat, i.e. a mixture of a large number of free amino acids. These substances could not be detected in the serum even if the blood was taken shortly after injection. If, however, blood taken after injection was boiled, these products became completely detectable. In experiments *in vitro* it was also established that amino acids, when added to blood, are quickly absorbed by the erythrocytes and released by boiling

or haemolysis caused by other means. Similar results were obtained in experiments *in vivo* and *in vitro* with inactivated diphtheria toxin.

The work of Zbarskii (1925a, b) and Zbarskii and Mikhlin (1925) showed that the erythrocytes of different kinds of animals vary in their ability to bind the products of protein decomposition.

Zbarskii (1925b) established that rabbit erythrocytes also absorb quinine, which, on adsorption by the erythrocytes, prevents the absorption by them of the products of protein decomposition. On this basis he came to the conclusion that the adsorption of amino acids and quinine is achieved by the same active sites and that erythrocytes have a closer "affinity" for quinine than for amino acids. Yakovlev (1938, 1939a, b, 1940a, b) showed convincingly that the amount of amino acids in erythrocytes is about twice as great as in the plasma, and that capillary-active substances as camphor, heptane, xylol, toluol, benzol, aniline, nitrobenzol, amyl alcohol and acetone force out of the erythrocytes the amino acids adsorbed by the colloids of these cells.

The adsorption of amino acids *in vitro* by erythrocytes has been observed by many workers. Thus, Häusler (1926) studied the absorption of glycine and alanine by washed human and cattle erythrocytes with varying amounts of these amino acids in the solution. Experiments were also done with whole blood. On heating the mixture for 1 hr at 37° the erythrocytes absorbed from 48 to 75 per cent of the amino acids depending on their concentration in the surrounding solution.

Kul'tyugin and Ivanovskii (1928), who carefully studied the process of the absorption of alanine by the erythrocytes of cats, dogs and cattle, point out the adsorptional mechanism of the uptake of amino acids by these cells. Tutkevich (1928a, b) came to the same conclusions as a result of his experiments with dog erythrocytes.

Many writers suppose that amino acids are adsorbed only on the surface of cells and do not penetrate inside them. However, the absorption of these substances by the formed elements of the blood does not strictly correspond to the adsorption isotherm, as was shown by Zbarskii (1925a), Glotzman (1931), Saichuk (1941) and others. On this basis London (1934), Miropol'skii (1938, 1950) and Zbarskii and Demin (1949) came to the conclusion that the uptake of an amino acid by erythrocytes is a more complex process than simple adsorption on the surface of cells. According to Zbarskii and Demin, the accumulation of an amino acid is "achieved both by adsorption and by diffusion within the erythrocytes with subsequent binding of part of the penetrating substances by the colloids of the red blood corpuscles" (p. 126).

Ussing (1943a) supposes that amino acids are not bound by erythrocytes. In a study of the distribution of amino acids in bovine blood with *in vitro* experiments he came to the conclusion that such monoamino acids as alanine, phenylalanine, leucine and tyrosine penetrate the blood cells and are distributed in equal proportions between the aqueous phases of the ery-

throcytes and the plasma. Dicarboxylic acids (glutamic and aspartic) do not penetrate erythrocytes or only extremely slowly. In this writer's opinion, the excess of amino nitrogen in the erythrocytes over the amount in the plasma is due to glutathione. The concentrations of the true (free) amino acids in the aqueous phases of the plasma and the erythrocytes are the same. However, Ussing's conclusions were not upheld by later work (Zbarskii and Kulakova, 1948, Zbarskii and Demin, 1949, etc.).

It has been established by a number of workers that amino acids penetrate erythrocytes at different speeds and have different coefficients of distribution between the plasma and the formed elements of the blood (Zbarskii and Kulakova, 1948; Zbarskii and Demin, 1949; Christensen, Riggs and Ray, 1952, and others). Thus, Zbarskii and Kulakova (1948) found that, on the introduction of amino acids into dog blood, cysteine is accumulated in the erythrocytes, and on the introduction of alanine in small doses its concentration in the erythrocytes is greater than in the plasma, while on the introduction of large doses, on the contrary, there is more in the plasma than in the erythrocytes; that leucine introduced into the blood is distributed equally between the erythrocytes and the plasma, while glycine at all doses remained in the plasma in greater concentrations than in the erythrocytes. Christensen *et al.* (1952) observed that in freshly isolated duck erythrocytes the concentration of glycine is 3–5 times (about 2 mm per kg of cell water) higher than in the plasma, that of alanine 1·6 times higher. When the cells are washed in salt solution the glycine slowly comes out of them. If the duck erythrocytes are placed in a 1 mm solution of glycine, the glycine penetrates the cells (0·4 mm glycine in the plasma); in this case equilibrium is attained inside of 1 hr. The same is observed if glycine is added to undiluted blood. Keeping blood at low temperatures causes the glycine to emerge from the erythrocytes. It passes back into the cells if the blood is then kept at normal temperature. According to these writers, the concentration of glutamic acid in human erythrocytes is three times greater than in the plasma, while that of glycine and alanine is 1·6 times greater. In experiments with duck erythrocytes it was shown that the dependence of the glycine concentration in the cells (calculated relative to the intracellular water) on its concentration in the medium has an adsorptional character at low concentrations, a linear dependence being observed at higher concentrations. At low concentrations the accumulation is very strongly marked, at higher, but weakly.

The passage of amino acids from erythrocytes into plasma and back again was convincingly demonstrated in experiments with dog blood by Zbarskii and Zubkova (1934) and with horse blood by Saichuk (1941). The latter succeeded in establishing that, on the reduction in the amino acid content of the blood, the loss is attributable principally to the erythrocytes, while the amount of amino nitrogen in the plasma remains almost constant.

The redistribution of amino acids between plasma and erythrocytes in serum in the whole organism was shown by London and Kochneva (1935),

Zbarskii (1936), Miropol'skii (1938, 1950), Fridlyand (1939) and others. A good impression of this work can be obtained from Table 33, which is taken from the work of Zbarskii (1936).

TABLE 33. THE DISTRIBUTION OF AMINO NITROGEN IN THE BLOOD OF VARIOUS VESSELS OF DOGS AFTER SHORT TERM FASTING AND "AT THE HEIGHT OF DIGESTION" OF ALBUMINOUS FOOD (from Zbarskii, 1936)

Time of taking blood	Place where blood taken	Amino nitrogen concentration (mg %)			Distribution coefficient
		whole blood	erythrocytes	plasma	
On an empty stomach	femoral artery	10.79	16.92	6.70	2.51
	portal vein	12.00	19.20	7.70	2.49
	hepatic vein	10.92	17.25	7.05	2.42
After 24 hr fasting	femoral artery	8.97	12.25	6.90	1.78
	portal vein	9.75	11.30	7.07	1.60
	hepatic vein	11.94	18.80	7.37	2.55
After 48 hr fasting	femoral artery	10.71	15.75	7.35	2.14
	portal vein	10.52	11.50	9.93	1.16
	hepatic vein	11.92	18.77	7.72	2.42
After 72 hr fasting	femoral artery	9.66	14.08	6.95	2.03
	portal vein	10.23	12.72	8.70	1.46
	hepatic vein	11.28	17.46	7.51	2.33
Same day, 4½ hr after feeding	femoral artery	11.71	19.17	6.93	2.77
	portal vein	12.80	22.15	7.09	3.15
	hepatic vein	11.30	17.45	7.67	2.28

It follows from the table that over the whole course of the experiment the amino nitrogen in the plasma of blood taken from various vessels is held at an almost constant level, whereas the amount of amino nitrogen in the erythrocytes varies very sharply. During fasting the passage of blood through the intestines will lead to loss of amino nitrogen by the erythrocytes into the plasma, while in the liver they are enriched in amino nitrogen.

For various pathological states (anaemia, scurvy, tuberculosis, cancer, disorder of the action of the endocrine glands, etc.) which lead to a change in the amino acid level in the blood, a sharp change is generally observed in the sorptional properties of the erythrocytes, as a result of which their amino nitrogen content changes considerably, while the amount in the plasma remains more or less constant (see Zbarskii and Demin, 1949).

Born and Gillson (1957, 1958) studied the permeability of human thrombocytes to 5-hydroxytryptamine and the effect on this process of 2,4-dinitrophenol. It was found that dinitrophenol in a concentration of  $1 \times 10^{-5} M$  caused an increase, and in a concentration of  $1 \times 10^{-3} M$ , on the other hand,

a decrease in the rate of penetration of hydroxytryptamine into the thrombocytes. An increase in the potassium ion content of the solution was observed to be accompanied by an increase in the rate of penetration of hydroxytryptamine into the platelets.

The picture of the distribution of polypeptides between the formed elements and the plasma of the blood is the same as that for amino acids. The only difference is that excess of polypeptide concentration in the cells over the concentration in the plasma is still more sharply marked than in the case of amino acids (Zbarskii and Demin, 1949; Miropol'skii, 1950, and others).

Thus we see from the above cited work that in the distribution of amino acids between the formed elements of the blood and the medium surrounding them the adsorptive binding of these substances by the cell colloids is of vital significance: it ensures the predominance of their concentration in the blood elements over their concentration in the plasma. The failure of amino acid absorption by erythrocytes to correspond to the adsorption isotherm shows that in this process the other sorptional factors (solubility and chemical binding) also play a part, as happens in the distribution of non-electrolytes.

TABLE 34. THE AMOUNT OF AMINO NITROGEN IN THE ORGANS OF CATS AND DOGS  
(from Miropol'skii, 1950)

Organs	Amount of amino nitrogen (mg%)	
	cat fasting	dog after food
Liver	78.8; 75.5	113.0
Kidney, whole	78.5; 82.0	84.3
Kidney, cortex	68.0	90.8
Kidney, medulla	104.0	89.2
Pancreas	79.2; 84.4	81.2
Spleen	89.4	118.7
Intestine, small	75.2; 76.3	87.8
Intestine, large	--	73.5
Lungs	79.2; 77.6	71.7
Muscles (skeletal)	77.0; 64.9	61.5
Heart	81.0; 83.0	69.5
Brain	60.4	53.2
Blood	10.5	10.4

*Muscle and nerve fibres, cells of other tissues and organs, micro-organisms.* Normally the distribution of amino nitrogen between the organs of animals and the bathing fluid is generally the same as between the formed elements and the plasma of the blood. The difference between the

amino acid concentration in the organs and the plasma is, however, many times greater than the difference between the amount of amino nitrogen in the erythrocytes and the plasma.

This problem has been studied meticulously by Miropol'skii (1950). Certain of his results are shown in Table 34. They show that the concentration of amino nitrogen in the organs varies in a cat from 60 to 104 mg per cent and in a dog from 53 to 118 mg per cent, whereas there is very much less in their blood:—10·5 mg per cent in the case of the cat and 10·3 mg per cent for the dog. If we consider that the amino acid concentration in the blood plasma is about 1·5–2 times less than in the erythrocytes, the difference between the amino acid concentrations in the organs and the bathing fluid then will be even greater.

TABLE 35. THE AMINO NITROGEN CONTENT OF THE ORGANS AND BLOOD OF VARIOUS ANIMALS  
(after Fedorova and Konikova, 1950)

Subject	Amount of amino nitrogen (mg per g of tissue)			
	liver	kidney	spleen	blood
Rats	0·38	0·45	0·36	0·21
Rabbits	0·42	0·42	0·66	0·19
Frogs	0·36	0·38	—	0·22

Fedorova and Konikova (1950), using colorimetric techniques, obtained somewhat different and less widely varying figures in experiments with rats, rabbits and frogs which had been fasting for 12 hr; these figures also indicate a considerable preponderance of the amount of amino nitrogen in the organs over their content in the blood and, consequently, a still greater preponderance over the content in the plasma. These results are shown in Table 35.

Using the method of dispersion chromatography on paper, they found that the total amino nitrogen in the organs mentioned in the table is composed of the nitrogen of the following amino acids: aspartic, glutamic, cysteine, glycine, serine, alanine, methionine, arginine and phenylalanine. There are probably other amino acids present apart from these nine.

Ussing (1943b), studying the distribution of amino acids between the blood plasma and the organs of guinea pigs, came to the conclusion that only glutamic acid is present in the cells in larger amounts than in the plasma, and that a number of other amino acids are distributed between the aqueous phases of the organs and the plasma in varying amounts. As proof he adduces the following fact. If leucine is introduced into the blood of an animal, then its concentration in the tissues is not increased more than it is in the blood.

In Ussing's opinion, the nitrogen of the tissues and the organs, like that of the erythrocytes, is obtained chiefly from the nitrogen of glutathione and, possibly, other lower peptides, and not from the amino acids. The results of other workers do not, however, agree with this. Thus, Hamilton and Van Slyke (1943), Christiansen and Lynch (1946), Zbarskii and Kulakova (1948) and others showed that because of the corrections for glutathione nitrogen there is more amino nitrogen in the cells than in the plasma, a preponderance which cannot be due to the glutamic acid alone, although the amount of this acid in the cells is 30–50 times greater than in the plasma, as is shown by the figures in Table 36. This table shows that cystine and cysteine also predominate in the cells.

TABLE 36. THE AMINO NITROGEN CONTENT OF THE ORGANS AND BLOOD PLASMA OF GUINEA PIGS. AMINO NITROGEN IN MG PER CENT (from Ussing, 1943 b)

Substance	Liver	Muscle	Kidney	Plasma
Total nitrogen <sup>(1)</sup>	45	45	+	5
Glutathione	30	10 <sup>(2)</sup>	20	0
Glutamic acid	5	16 <sup>(2)</sup>	9	0·3
Arginine	—	1	+	—
Histidine	—	—	—	—
Cystine + cysteine	0·7	0·2	0·1	0·1
Tyrosine	0·12	0·11	0·22	0·12
Leucine + valine	0·8	0·6	0·9	0·7

<sup>(1)</sup> Nitrogen of amino acids from Van Slyke.

<sup>(2)</sup> Heart muscle.

Of great interest are analyses of the axoplasm of giant nerve fibres of certain representatives of crustacean and cephalopod molluscs. The amount of amino acids in the axoplasma of these animals is several times greater than in the blood plasma (Schmitt, Bear and Silber, 1939; Silber and Schmitt, 1940; Silber, 1941, and others).

The permeability of muscle fibres for amino acids was demonstrated long ago by Van Slyke and Meyer (1913). They showed that on the introduction into the blood of an animal of certain amino acids, the latter were distributed between the blood plasma and the formed elements and also between the plasma and the muscle fibres and the cells of other organs and tissues. In the muscles the writers found the amino acids in an unchanged state, in a quantity 5–10 times in excess of that in the surrounding medium. Later, these observations were confirmed by experiments on whole organisms and on isolated organs of a number of animals (Cohen, 1939; Christensen, Streicher and Elbinger, 1948; Christensen and Streicher, 1949; Manchester and Young, 1960, and others).

Thus, there can be no doubt about the very considerable preponderance of the amino acid concentration in the organs over their concentration in

the plasma. This preponderance shows that the cells which go to make up the organs and tissues have great powers of accumulation with respect to amino acids.

In Miropol'skii's opinion, about half the plasma amino acids are in the dissolved state, the remaining half being adsorbed by the proteins. He also suggests that the total amino acid in the cells is partly dissolved in water and partly adsorbed by the protoplasm colloids, supposing that the amino acid concentration in the protoplasm water is the same as in the water of the blood plasma. Miropol'skii supposes that thanks to the change in the adsorptional properties of the proteins of the protoplasm the amino acids in the cell can pass into diffusible form and thence into the adsorbed state, as a result of which they either leave or enter the cell. In support of such a mechanism of the redistribution of amino acids and other substances between cells and medium there are, as we shall see later, fairly weighty arguments. However, Miropol'skii's assumption that the amino acid concentration is the same in the aqueous phases of the cells and the blood plasma is not borne out by experiment.

Thus, I have studied the distribution of  $\alpha$ -alanine between frog gastrocnemius muscles placed for various lengths of time in solutions of this substance made up in Ringer's solution (Troshin, 1951d).

The experimental procedure was in principle the same as that used in the preceding experiments with non-electrolytes. The amounts of alanine added to the medium and remaining in it at the end of the experiment were determined by Sørensen's method. As the experiments were done with small amino acid concentrations, all the solutions were made up in a Ringer's solution of complete composition. The loss of water by the muscles even at the highest concentrations did not exceed 5–6 g per 100 g of muscle water.

For comparison, parallel experiments were done with dead muscles. These were killed by a mixture of 96 per cent alcohol with double Ringer's solution in proportions of 1:1. The muscles were placed in this solution for 2 hr, after which they were washed in Ringer's solution for 6 hr till all traces of alcohol had been removed. After washing, the amount of water in the dead cells was the same as in the live (about 80 per cent).

In the first group of experiments the subject was the dynamics of the penetration of alanine into muscles with time until the establishment of diffusion equilibrium. The results are given in Figs. 62 and 63.

The curves show that equilibrium between the alanine solution and the muscles (alive and dead) is achieved after 2–3 hr. In conditions of diffusion equilibrium the amino acid concentration in the dead muscles was about the same as in the equilibrated solution ( $Q = 0.92-1.02$ ), while under the same conditions in the live muscles the alanine concentration could be either greater or less than in the medium: depending on the amino acid concentration in the medium,  $Q$  varied from 0.42 to 1.32.

The second group of experiments was devoted to the dependence of the alanine concentration in the muscle on its concentration in the surrounding

equilibrated medium. These experiments lasted 3 hr, which was sufficient to allow the establishment of complete diffusion equilibrium between the external alanine solution and the muscles (see Figs. 62 and 63). The results of this group of experiments are shown graphically in Figs. 64–66.

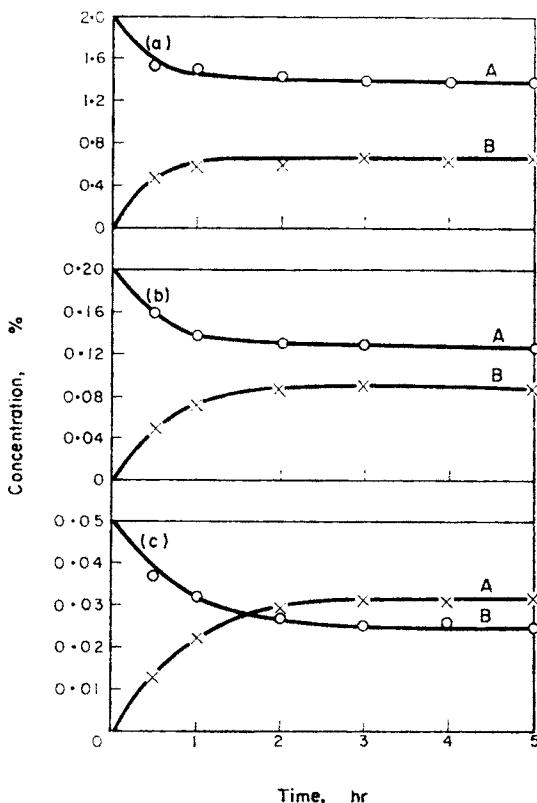


FIG. 62. The penetration of *d*-alanine into frog muscle fibres from the medium with time.

Initial concentrations of the alanine solutions: a—2 per cent, b—0.20 per cent, c—0.05 per cent.

A—diminution of the alanine in the medium, B—absorption of the alanine by the muscles.

As can be seen from the figures, a decrease in amino acid concentration in the surrounding medium is observed to be accompanied by an increase in the percentage absorption of amino acid both by the muscles as a whole and by the muscle fibres. In Fig. 64 it can be seen that, when  $C_s = 0.025$  per cent,  $C_c = 0.033$  and  $Q = 1.32$ , so that there is 32 per cent more amino acid in the fibres than in the medium, while when  $C_s = 0.491$  per cent,  $C_c = 0.250$  and  $Q = 0.45$ , i.e., there is now 55 per cent less of this substance in the fibres

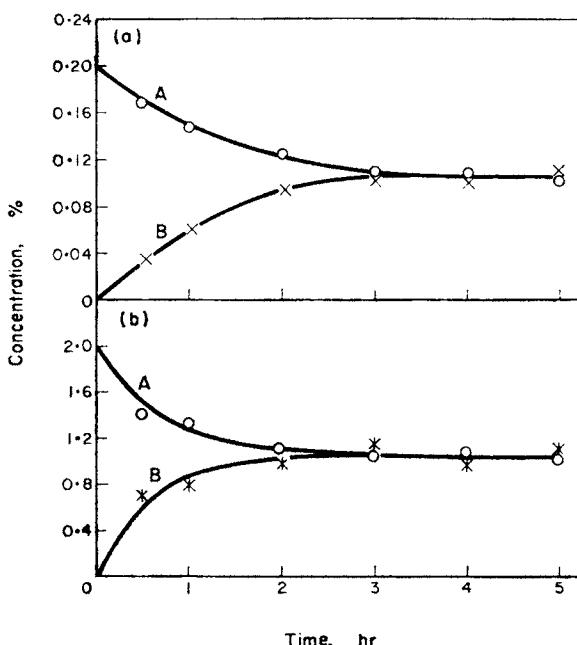


FIG. 63. The penetration of alanine into dead frog muscle fibres with time.  
Initial alanine concentrations: a—0.20 per cent, b—2.0 per cent; A—the diminution of the alanine in the medium, B—the absorption of alanine by the muscles.

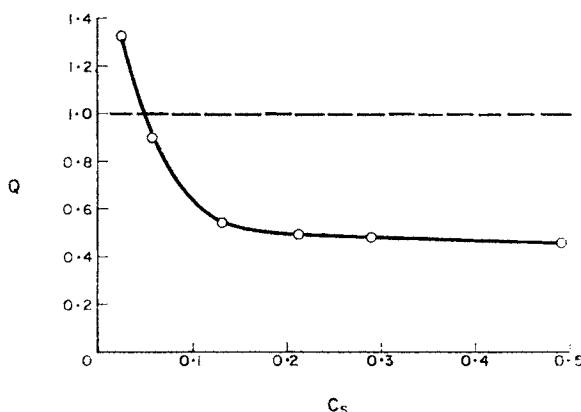


FIG. 64. The dependence of the coefficient  $Q$  on the alanine concentration in the medium ( $C_s$ , in per cent) when it is distributed between frog muscle fibres and a surrounding external solution.

than there is in the medium. The path of the curve shows that only when  $C_s = 0.049$  per cent  $Q = 1$ : if  $C_s$  is smaller,  $Q$  is greater than unity, while if  $C_s$  is greater, then  $Q$  is less than unity (there is less alanine in the muscle fibres than in the medium).

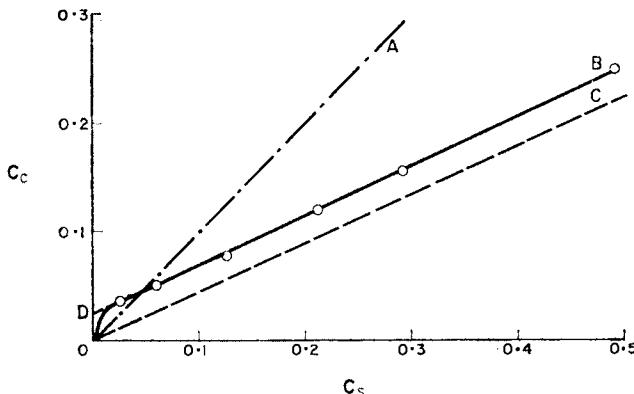


FIG. 65. The dependence of the alanine concentration in the muscle fibres ( $C_c$ ) on its concentration in the equilibrated medium ( $C_s$ ).

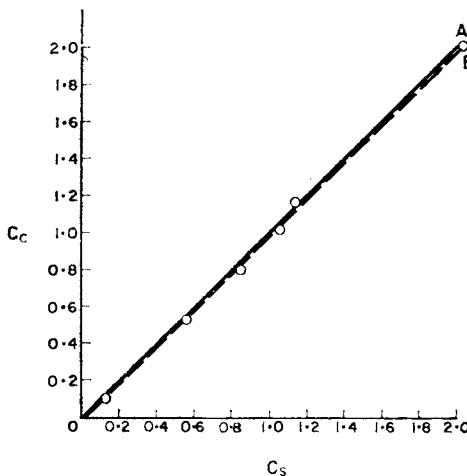


FIG. 66. The dependence of the alanine concentration in dead muscles ( $C_c$ ) on its concentration in the surrounding medium ( $C_s$ ).

Thus, similar results were obtained in the alanine experiments as were obtained in those with sugars, creatinine and urea.

In experiments with dead muscles nothing of the kind is found. Here, at all the alanine concentrations tested its concentration in the muscles is about the same as in the medium.

Thus, the mechanism of the distribution of an amino acid between live muscles and the medium is fundamentally different from that between dead muscles and medium. This difference can be portrayed graphically. In Fig. 65 the curve OB shows the dependence of the  $\alpha$ -alanine concentration in the muscle fibres on its concentration in the surrounding medium. In Fig. 66, the same dependence is shown by the straight line OB for dead muscles. As can be seen from the figures, there is over the whole range of concentrations tested a linear relation between  $C_c$  and  $C_s$  which can be expressed by the formula  $C_s = C_c K + A_\infty$ , where  $A_\infty$  (see Fig. 55) is equal to the intercept OD on the ordinate, or to 0.029 g of alanine per 28.6 g of dry residue of muscle corresponding to 100 ml of intracellular water. The line OC parallel to DB, which reflects the dependence of the concentration of dissolved alanine in the water of the muscle fibres  $C$  on  $C_s$ , obeys the law  $C = C_s K$ , where  $K = 0.40$ . In other words, there is 60 per cent less alanine dissolved in the muscle fibres than in the water of the surrounding medium.

The line OB, which reflects the distribution of alanine between dead muscles and medium (Fig. 66), also obeys the above formula, except that now the constants are quite different:  $A_\infty = 0$  and  $K = 0.95$ . This means that  $\alpha$ -alanine is not adsorbed at all by dead muscles, while its solubility in them is only 5 per cent less than in the water of the surrounding medium. This is almost equal to the amount of "bound" water which is normally found for dead colloids (Pauli and Val'ko, 1936; Hill, 1935, etc.).

This conclusion that dead muscle does not adsorb alanine at all confirms facts known long since from the study of the distribution of amino acids between cells and medium. Thus, on the boiling of erythrocytes, the amino acids adsorbed on them, as has already been noted, pass entirely into solution (Zbarskii and Demin, 1949).

Thus, the distribution of alanine between live muscle and the medium surrounding it is determined by two fundamental factors: the lowered solubility of alanine in the protoplasm and its adsorption by the cell colloids. It is the combination of these two factors that results in the relation between  $C_c$  and  $C_s$  described above.

On the basis of the experimental data obtained it may be concluded that alanine quickly penetrates live muscle fibres and is distributed between the muscles and the medium in accordance with its solubility in the protoplasm and the adsorption of this amphotelyte by the colloids of the live protoplasm.

Thus, amino acids which pass into a cell from the surrounding medium can exist in the protoplasm in the dissolved and in the adsorptionally bound state. In the process of metabolism they are transformed inside the cell into other chemical compounds which, as a whole or in individual groups, go to make up the cell proteins, and so on.

In recent years the isotopic method of studying protein exchange has been widely used. This method has made it possible to determine the speed of renewal of the proteins of an organism, to observe the dynamics of their

breakdown and synthesis, to trace the inclusion into the cell proteins of amino acids from the surrounding medium and their transformation into other substances. Labelled amino acids are introduced into the organism with its food or by injection into the blood stream and their subsequent fate is followed. At present the labelling of amino acids is done with isotopes of all the elements which enter into their composition, nitrogen  $^{15}\text{N}$ , carbon  $^{11}\text{C}$ ,  $^{13}\text{C}$  and  $^{14}\text{C}$ , hydrogen  $^2\text{H}$ , etc. Frequently amino acids are labelled not by one element but by several elements of the same molecule. It is then possible to follow the inclusion of amino acids into various proteins and to observe the transformation and ultimate destiny of its individual components.

The literature on protein turnover as studied by means of labelled atoms is very extensive. A considerable part has been reviewed in the articles of Schoenheimer (1942), Konikova (1948), Borsook (1950), in Khesin's book (1960) and in other places.

TABLE 37. THE RATE OF PROTEIN RENEWAL IN VARIOUS RAT ORGANS  
(from Orekhovich, Konikova *et al.*, 1950)

Organs	Surplus at.% of deuterium after introduction of $\text{D}_2\text{O}$	% protein renewal
Liver	0.232	23.2
Intestines	0.170	17.0
Spleen	0.167	16.7
Kidneys	0.162	16.2
Stomach	0.137	13.7
Heart	0.136	13.6
Lungs	0.115	11.5
Brain	0.112	11.2

The rate of renewal of the proteins of fully grown organisms as determined by this method is strikingly high. Thus, Springson and Rittenberg (1949) gave  $^{15}\text{N}$ -glycine to rats with their food and found from the speed with which it was included in the proteins of the organs that in rats the period of the decomposition of half of the total protein is 17 days, of the protein of the internal organs and the plasma 6–7 days, of the proteins of the body 21 days. For humans the corresponding period for the total protein was found to be 80 days, for the proteins of the liver and the plasma 10 days, for the proteins of the brain, lungs, ribs, skin and the greater part of the muscles 158 days.

In these experiments the renewal of the proteins was studied from the inclusion into them of a single amino acid. However, about the same rate of renewal is observed when several labelled amino acids are followed (Borsook, 1950). Orekhovich, Konikova and others (1950) studied the overall rate

of renewal of the proteins of various organs and tissues in rats. They introduced heavy water ( $D_2O$ ) into the blood of the rats keeping the concentration in the body fluids at 1 per cent for 12 days. From the amount of deuterium included in the protein molecules they estimated the rate of the total renewal of the proteins. It was found that the rate of renewal varied for different organs, just as the rate varied for different proteins in the same organ: these findings are illustrated by the figures in Tables 37 and 38.

TABLE 38. THE RATE OF RENEWAL OF INDIVIDUAL PROTEIN FRACTIONS IN VARIOUS RAT ORGANS (from Orekhovich, Konikova *et al.*, 1950)

Proteins	Excess at.% of deuterium	% renewal
Blood proteins	0.181	18.1
Blood globulins	0.148	14.8
Liver globulins	0.137	13.7
Skin globulins	0.138	13.8
Skin albumins and globulins	0.177	17.7
Skin collagen	0.132	13.2
Skin procollagen	0.123	12.3
Muscle proteins	0.101	10.1
Myogen	0.089	8.9

The results show that in the body of a full grown organism in the absence of an increasing mass of protein continuous renewal processes take place at great speed. This eliminates the possibility of the cells being impermeable for amino acids. And in fact, experiments with labelled amino acids have shown that they penetrate cells quickly.

Thus Tarver and Reinhardt (1947) introduced  $^{35}S$ -methionine intravenously into dogs. Two hours after introduction they were able to detect it in all the internal organs, but particularly in the intestines. After 5 hr the increase in the amount of labelled amino acid in the organs by comparison with the state after 2 hr was quite insignificant.

Greenberg and Winnick (1948) introduced into the blood of rats 25 mg of glycine labelled with  $^{14}C$  in the carboxyl group (activity of the glycine 25,000 counts per min per mg) and obtained the results shown in Table 39 for the speed of inclusion of glycine in the proteins of the various organs.

Similar results were obtained by Winnick, Friedberg and Greenberg (1948) in experiments with rats when  $^{14}C$ -tyrosine was introduced into the blood, and by Friedberg, Tarver and Greenberg (1948) in experiments with rats and dogs in which  $^{35}S$ -methionine was introduced intravenously.

According to the calculations of Borsook (1950) many amino acids on introduction into the blood of an animal pass very quickly into the cells

TABLE 39. THE RELATIVE SPEED OF INCLUSION AND LOSS OF  $^{14}\text{C}$  FOR VARIOUS RAT ORGANS AFTER THE INTRODUCTION INTO THE BLOOD OF GLYCINE LABELLED WITH  $^{14}\text{C}$   
 (from Greenberg and Winnick, 1948)

Organ	Activity in % of introduced dose of $^{14}\text{C}$ per g of protein after				
	15 min	6 hr	18 hr	3 days	5 days
Mucous membrane of intestines	0.3	3.7	3.25	1.35	0.95
Bone marrow	0.2	2.15	2.2	1.4	0.9
Liver	0.25	2.05	1.35	1.2	0.85
Kidneys	0.15	1.95	1.3	1.1	0.95
Plasma	0.05	1.8	1.6	0.95	0.75
Spleen	0.05	1.45	1.25	0.8	0.6
Lungs	0.15	1.25	1.1	0.85	0.65
Testicles	0.05	0.5	0.5	0.45	0.4
Muscles	0.0	0.15	0.2	0.2	0.2
Erythrocytes	0.0	0.15	0.15	0.2	0.25
Brain	0.0	0.1	0.15	0.1	0.1

of the various organs and become included in the proteins. This can be seen from Table 40.

The results obtained in experiments with labelled amino acids indicate the great speed with which they are included in the cell proteins. The process is so quickly accomplished that in 30–40 min equilibrium is achieved between the included labelled amino acids and free unlabelled amino acids: this follows from Table 40 and is illustrated in many other places as well.

TABLE 40. THE SPEED OF INCLUSION OF AMINO ACIDS LABELLED WITH  $^{14}\text{C}$  INTO THE PROTEINS OF THE INTERNAL ORGANS OF RATS (from Borsook, 1950)

Time (min)	Amino acids (in meqv. per kg live weight)			
	glycine	<i>l</i> -histidine	<i>l</i> -leucine	<i>l</i> -lysine
	Introduced			
	0.5	0.25	0.25	0.25
Included (in meqv. per g of protein)				
10	0.65	0.64	1.4	0.3
20	2.0	1.3	3.0	2.0
30	3.1	2.9	3.6	2.2
60	3.2	3.1	3.6	2.6
120	2.8	2.8	4.0	2.4
240	2.8	3.1	4.3	2.8

Only an approximate impression of the speed with which the amino acids penetrate the cells can be obtained from the speed with which they are included in the proteins of the organs. In any case this speed cannot be less than the speed of inclusion into the cell proteins.

Many authors have found by the method of labelled amino acids that the latter are included in the composition of the proteins of isolated organs with approximately the same speed as when they are introduced into the blood of the live animal. Thus Borsook and his colleagues (1949) found that guinea pig, rabbit and rat bone marrow cells outside the organism include amino acids into their proteins at an unchanged rate. Different labelled amino acids are included into the bone marrow cell proteins at different rates: glycine at  $1.3 \times 10^{-3}$ , *L*-histidine at  $1.1 \times 10^{-3}$ , *L*-leucine at  $2.2 \times 10^{-3}$  and *L*-lysine at  $1.5 \times 10^{-3}$  equivalents per g of protein per hr. Under the same conditions the above amino acids are included into the proteins of rat diaphragms at a rate 3–5 times less than in the case of bone marrow proteins. These workers established that cells which have been damaged (by ether, distilled water, mechanical damage, freezing and thawing, etc.) are not capable of incorporating amino acids into proteins.

It was also found that the optimal condition for the inclusion of amino acids into proteins occurred when the concentration of amino acids in the medium is the same as in the blood. The speed of inclusion of amino acids in the proteins grows rapidly with increasing concentration. However, when the concentration rises above the level in the plasma, the speed of inclusion increases very slowly or not at all. A similar law was found by Kit and Greenberg (1952) in a study of the inclusion of labelled threonine and valine into the proteins of rat liver.

In the literature there is an enormous amount of information to show that amino acids and their derivatives penetrate the cells of different organs at different speeds. This is undoubtedly because the processes of cellular metabolism in the various organs proceed at different rates. In a recent work Halpern, Neven and Wilson (1959) found that on the introduction of  $^{14}\text{C}$ -histamine into the blood of dogs it was no longer detectable in the blood plasma after a few seconds—it is all found in the cells and is quickly decomposed there. In order of decreasing speed of penetration of histamine into their cells the organs can be placed in the following series: kidney, liver, lungs, small intestine, large intestine, spleen, stomach, skin, muscles.

The same method has been used to show that these substances also penetrate very rapidly into the cells of micro-organisms. Thus Webb, Friedberg and Marshall (1951) found that in an atmosphere of oxygen the yeast *Torula utilis* quickly absorbed from the surrounding liquid labelled glycine and includes it in the protein molecules. This process was observed to be at its fastest at  $35\text{--}36^\circ$ ; at  $10^\circ$  it was very slow and at  $50^\circ$  almost at a standstill. A change in the concentration of hydrogen ions in the medium towards either side of pH = 6.0 inhibited this process very strongly. On exposure to

a 2·5 per cent solution of phenol for 2 hr the inclusion of glycine into yeast proteins was almost completely inhibited. On the basis of these experiments the writers came to the conclusion that the inclusion of glycine in the cell proteins is an enzymatic process of protein synthesis. The same conclusion has been reached by many other writers as well (see Borsook, 1950, etc.).

Rapid absorption of  $^{35}\text{S}$ -methionine by resting cells of *Escherichia coli* was observed by Melchior *et al.* (1948). Cowie and Roberts (1954) found that these cells are highly permeable for many substances, including cystine, glutamine, methionine and glutathione. In the opinion of these writers the substances diffuse out of the medium into these cells until their concentration in the whole cell water reaches the external concentration. However, Cohen and Rickenberg (1955) disagree. By using radioactive amino acids they were able to detect in cells of *Esch. coli* the presence of acceptors which specifically reversibly fix the amino acids of the nutrient medium. This process obeys the laws of adsorption and represents, so they consider, a preliminary stage in the inclusion of amino acids in proteins. According to Britten (1956), the cells of *Esch. coli* can accumulate amino acids in great quantities, forming an amino acid metabolic pool.

Gale (1947) studied the permeability of the cells of *Streptococcus faecalis* for a number of amino acids. He found that lysine can accumulate in large amounts in these bacteria, so that its concentration in the cells at small concentrations in the medium exceeds the external by more than 20 times. This excess falls with increasing lysine concentration in the medium. Taylor (1947) found that 13 Gram-positive forms of bacteria and three types of yeast are also capable of absorbing lysine and glutamic acid against the concentration gradient, while in 11 Gram-positive forms the absorbing power is low. Gale (1953, 1954), in a discussion of numerous results, came to the conclusion that amino acids can penetrate cells by simple diffusion and are bound within the cell in some way, thereupon passing into a non-diffusible form. If this is indeed so, then the accumulation of amino acids by cells is not an active process, because in this case there is no place for the passage of amino acids into cells against a concentration gradient.

We have already considered results which indicate that a substance which somehow or other penetrates a cell, can exist in the protoplasm in various states—the free (dissolved) state and in the adsorptionally or some otherwise bound state with the cell structures. In this connection the work of Cowie and McClure (1959), Halvorson and Cowie (1961) and others is of great interest. They found in yeast cells two "amino acid pools" differing from one another both chemically and functionally. The first "expandable" pool ensures the accumulation within the cell of amino acids up to a level exceeding their concentration in the external medium. The size of this pool depends on the external amino acid concentration. Its components exchange with the amino acids of the medium, they are extracted by cold water and so on. The amino acids of the first pool can pass reversibly into the second

pool—the “internal pool”. This is present in growing cells in which the amino acids are synthesised from carbohydrates and mineral nitrogen in the region of the first pool. What takes place in the second pool is the transformation of certain amino acids into others and their inclusion in the proteins. Exogenous amino acids or components of the first pool have no effect on the size of the second pool and the inclusion of the amino acids of this pool into proteins. The amino acids of the second pool cannot be extracted with cold trichlor acetic acid.

Amino acids also penetrate plant cells very quickly. Thus, labelled aspartic acid penetrates all the cells of lupin seedlings 2·5–5 times more quickly than labelled glucose (Nelson, Krotkov and Reed, 1933).

But even greater is the speed with which amino acids and certain of their derivatives penetrate tumour cells, accumulating there in large amounts. Thus, Christensen and Riggs (1952) found that in a liquid containing Ehrlich ascites tumour cells the amino acid concentration is the same or even lower than in the blood plasma, whereas the concentration of amino acids (glycine, alanine, glutamic acid etc.) in the tumour cells themselves is considerably higher. These amino acids can freely be extracted from the cells.

Individual amino acids added to the suspending fluid are concentrated in the tumour cells with an activity not inherent in the cells of mammalian tissues in experiments outside the organism. The swelling of the cells connected with a large accumulation of glycine indicates, in the opinion of these writers, that the accumulated amino acids are in the cells in an osmotically active form. The concentration of amino acids is observed most strongly at temperatures from 20° to 38°, there being none to be seen at 1° and 51°. The accumulation of amino acids is strongly depressed by anoxia, cyanide, dinitrophenol and arsenate, which is not seen in experiments with erythrocytes (Christensen, Riggs and Ray, 1952). A 40 mm concentration of potassium in the medium severely depresses the absorption of glycine by tumour cells. If there is a still greater concentration of glycine in the medium, then potassium is seen to emerge from the cells and sodium to pass into them.

In Eagle's laboratory (Eagle, 1955, 1958a, b, 1959, 1960; Eagle *et al.*, 1958, 1959; Levintow *et al.*, 1957; Salzman *et al.*, 1958, 1959; Piez and Eagle, 1958) a detailed study has been made of the permeability of cultured normal and tumour cells of humans for a large number of amino acids and also for pyruvic acid and pyrimidine bases. It was found that certain amino acids are accumulated in very large amounts by these cells, so that their concentration inside the cells becomes many times greater than in the surrounding medium.

Heinz (1954), studying the kinetics of the penetration of  $^{14}\text{C}$ -glycine into ascites cells, found that the maximum speed of penetration of this amino acid is  $45 \times 10^{-6}$  at 28° and  $57 \times 10^{-6}$  mm/mg dry weight per minute at 37°. The initial speed of penetration of glycine into the cells falls with increasing concentration of amino acid in the medium—it changes according to the

Michaelis-Menten rule, which contradicts Fick's law. The reason for this phenomenon may be that the amino acid entering the cell is somehow bound by the cell structures. In the opposite case, if the amino acids entering the cell stay there in the free form and can accumulate, one must admit the existence of a membrane mechanism of active transport of the amino acids. The writer proves that it is just this mechanism that occurs. Similar conclusions were reached by Jacquez (1959) in a study of the kinetics of the penetration of  $\alpha$ -methyltryptophane into Ehrlich ascites tumour cells. This tryptophane derivative penetrates the cells quickly and accumulates in them, but, like all  $\alpha$ -methylamino acids, is not utilised by the cells (see also: Jacquez, 1957, 1961 a, b; Jacquez and Hutchison, 1959).

Thus it has been proved by chemical analysis and by the method of labelled atoms that amino acids penetrate into a wide variety of live cells, in which they can accumulate in large quantities.

## 2. The Permeability of Cells for Ascorbic Acid

*Formed elements of the blood.* The permeability of cells for ascorbic acid and its oxidation products has been studied in great detail by many workers.

About 1 mg per cent of ascorbic acid is found in the blood of healthy humans, there being approximately an equal quantity of this substance per unit volume of plasma and erythrocytes. Leucocytes contain many more times vitamin C relatively than the plasma and erythrocytes. This can be seen from the data set out in Table 41.

TABLE 41. THE DISTRIBUTION OF ASCORBIC ACID IN HUMAN BLOOD

(in mg per 100 ml) (Stephens and Hawley, 1936)

No.	Whole blood	Plasma	Erythrocytes	Leucocytes
1	0.86	1.12	0.84	11.80
2	1.10	1.10	0.94	8.65
3	1.27	1.19	1.19	13.15
4	1.48	1.34	1.08	26.40

According to Stephens and Hawley (1936), the amount of vitamin C in the blood of leukemic patients is 2–5 times greater than normal; but this increase is not due to any enrichment of the ascorbic acid content in the plasma or formed elements of the blood, but to the increased number of leucocytes.

There are differences of opinion among workers about the permeability of erythrocytes to ascorbic acid. Some writers assert that these cells are impermeable to ascorbic acid (Borsook *et al.*, 1937, etc.). However, the majority of research workers conclude otherwise (Butler and Cushman, 1940; Heinemann, 1941; Roe *et al.*, 1947; Sargent, 1947, and others).

The permeability of the erythrocytes of various animals and humans to ascorbic acid and its oxidised form—dehydroascorbic acid—was the subject of some detailed *in vitro* experiments by Panteleyeva (1950). She established that the erythrocytes of humans, rabbits, cats and horses are permeable to ascorbic acid, whereas those of dogs and geese are not. Dehydroascorbic acid penetrates very quickly into the erythrocytes of all the above mentioned animals and humans, and also into the erythrocytes of pigs.

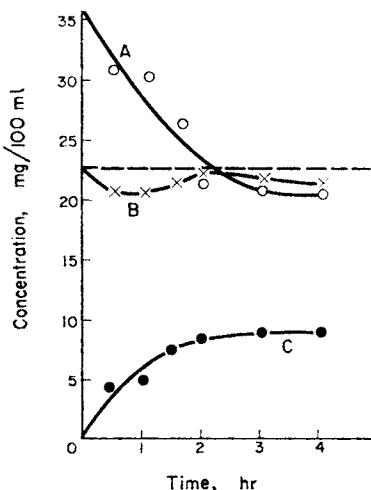


FIG. 67. The distribution of ascorbic acid between human erythrocytes and blood serum with time (from Panteleyeva, 1950).

A—in the liquid part of the blood (in the medium), B—in the suspension,  
C—in the erythrocytes.

Panteleyeva's data on the distribution of these substances between the erythrocytes and serum are of great interest. She established that ascorbic acid when added to human blood penetrates inside the erythrocytes, reaches in 2 hr a concentration level a third that in the serum and then for a long time remains unchanged as long as its content in the whole blood is unchanged (Fig. 67). She obtained similar results with the blood of rabbits, cats and horses. In these cases the coefficient of distribution  $Q$  was always considerably less than unity (0.22–0.26).

In contrast to ascorbic acid, its dehydro form when added to human blood quickly penetrates the erythrocytes and accumulates in them in a concentration greater than that in the serum. Its distribution coefficient increases very quickly with time, and is the greater, the less the concentration of the dehydro form in the serum, as is indicated by the data in Table 42.

Panteleyeva also studied the distribution of the dehydro form of vitamin C between the erythrocytes and plasma of various animals. Some of the results from this series of experiments are given in Table 43.

It can be seen from this table that the coefficient  $Q$  for all the animals mentioned is less than unity, varying within the limits 0·22–0·45.

It is possible that at lower concentrations the distribution coefficient would be greater than unity, as happens in the case of human erythrocytes (see Table 42).

*Cells of other tissues and organs.* It is well known that the various tissues and organs of animals contain different amounts of ascorbic acid.

TABLE 42. THE DISTRIBUTION OF THE DEHYDRO FORM OF ASCORBIC ACID (DAA) BETWEEN HUMAN SERUM AND ERYTHROCYTES IN DEPENDENCE ON THE CONCENTRATION OF THE DAA INTRODUCED INTO THE BLOOD. TEMPERATURE 37°C (from Panteleyeva, 1950)

No	DAA introduced (mg%)	DAA content (mg%)		$Q$
		plasma	erythrocytes	
After $\frac{1}{2}$ hr				
1	10·0	2·8	18·2	6·5
2	17·0	8·4	24·4	2·9
3	21·7	15·0	22·0	1·4
4	23·6	13·0	29·0	2·1
After 1 hr				
5	9·6	2·8	14·0	5·0
6	9·8	2·8	16·0	5·7
7	16·0	6·9	23·1	3·3
8	18·7	9·2	20·4	2·2

According to Lavrov (1947) and his colleagues, the amount of ascorbic acid in the organs of white rats is as follows (in mg per cent): liver—13·6–16·7; spleen—37·7–43·3; small intestine—24·4–28·8; kidney—8·3–10·2; suprarenals—318·4–404.

Tron and Tartakovskaya (1955) observed that the fibres of the crystalline lens of the eyes of large cattle, in whose protoplasm there is normally a large

TABLE 43. THE DISTRIBUTION OF DAA BETWEEN THE SERUM AND ERYTHROCYTES OF VARIOUS ANIMALS (after  $\frac{1}{2}$  hr at 37°). (From Panteleyeva, 1950)

Subject	DAA content (mg%)		$Q$
	serum	erythrocytes	
Cat	22·5	10·2	0·45
Pig	20·7	6·9	0·33
Horse	27·6	9·1	0·32
Dog	27·2	7·6	0·28
Rabbit	21·6	4·8	0·22
Goose	28·2	6·3	0·22

amount of ascorbic acid, slowly let this out into the surrounding medium if the lens is placed in a physiological solution. If the hormone of the parathyroid glands, parathormone, is added to the physiological solution, it increases the rate of loss of vitamin C from the lens fibres, while adrenaline has the opposite effect.

On the introduction of vitamin C into animal blood the various organs absorb it in different amounts (Table 44).

This table shows that the various organs absorb an amount of vitamin C such that its concentration in them is ten times that in the blood plasma.

TABLE 44. THE DISTRIBUTION OF ASCORBIC ACID BETWEEN THE PLASMA AND ORGANS  
(IN MG PER CENT) ON ITS INTRODUCTION INTO THE BLOOD OF GUINEA PIGS  
(from Klimov, 1951)

Plasma	Adrenals	Liver	Kidneys	Heart muscle	Brain
0.57	70.0	9.6	6.0	4.8	14.4
0.14	18.4	4.2	1.6	0.9	8.3
0.04	2.2	0.9	0.4	0.4	4.7

This excess is, evidently, due to the fact that the major part of the ascorbic acid in the cells is not in the free, but in the bound state. It has been established by many workers that ascorbic acid is principally bound with protein (ascorbinate) in the tissues of an organism. Under certain conditions this bond is broken down, and the vitamin C passes into the free form. According to Matusis (1947) and his colleagues up to 40 per cent of the ascorbic acid in the body of humans and animals is in the bound form (see Borsook, 1949; Eideman and Gordon, 1949).

The material cited above is convincing evidence that the distribution of vitamin C and its dehydro form between cells and medium is regulated by the same sorptional factors as regulate the distribution of amino acids, sugars, urea and other non-electrolytes.

### 3. The Permeability of Cells for Pyruvic Acid

The distribution of pyruvic acid between the formed elements of the blood and the plasma has been studied very meticulously in view of the important part which it plays in carbohydrate exchange. Shcherbatskaya (1939) found that there was about 4.45–4.53 mg per cent pyruvic acid in the blood of dogs 16–18 hr after feeding. There was 1.54–1.56 mg per cent in the plasma and 2.76–2.82 mg per cent in the erythrocytes. One hour after giving glucose by mouth the amount of sugar and pyruvic acid in the blood plasma increases almost twice, while the quantities of these substances in the erythrocytes

remain unchanged. One hour after the subcutaneous injection into dogs of adrenaline the amount of sugar in the plasma is increased by 93 per cent compared with the normal and in the erythrocytes by 25 per cent. In this period the concentration of pyruvic acid in the plasma increases by 174 per cent and that in the erythrocytes by 13 per cent. The subcutaneous introduction of insulin causes a reduction in the amount of sugar in the plasma by almost half, and by a third in the erythrocytes. In the same period the pyruvic acid concentration in the plasma remains almost unchanged, while there is a sharp increase in the concentration in the erythrocytes. These results are convincing proof that erythrocytes are permeable to glucose and pyruvic acid alike.

The permeability of erythrocytes to pyruvic acid was shown by Prokhorova (1950a, b) in experiments with dogs. She obtained results fundamentally similar to those of Shcherbatskaya on the introduction into the blood of the animal of adrenaline and insulin, and also on feeding with glucose. Further, Prokhorova showed that on the introduction into the blood of the sodium salt of pyruvic acid there is an increase in its concentration in the erythrocytes and the plasma. These experiments leave no room for doubt about the permeability of erythrocytes for pyruvic acid. Prokhorova also found a one and a half to three-fold excess of the pyruvic acid concentration in the erythrocytes over its concentration in the plasma. The cause of this, in her opinion, is to be found in the fact that "the level of pyruvic acid in the erythrocytes depends, first of all, on the intensity of its metabolism in the erythrocytes themselves, which is in its turn determined by the intensity of glycolysis and partly by the speed of penetration of pyruvic acid into the erythrocytes and back again" (1950b, pp. 212-3).

In experiments on angiotomised dogs Prokhorova (1936, 1950a) established that the muscle fibres are permeable for pyruvic acid, because, when muscular work is done, this substance is observed in increased quantities in the plasma of the blood that flows from the muscles. On the basis of her experiments the same may be said about the permeability of the cells of the liver, brain and intestine.

Analogous results were obtained by Ivanenko, Kazimirova and Prokhorova (1936). Further, these writers observed, besides the release of pyruvic acid into the blood by working muscles, increased absorption by the muscles of sugar and glycogen from the blood.

Thus, both in their normal conditions and in experiments outside the organism animal cells are permeable to pyruvic acid, just as they are permeable to sugars, amino acids and other substances; they can also accumulate it in considerable quantities.

#### 4. The Permeability of Cells for Uric Acid

Normally uric acid is constantly present in the blood of animals and humans. The work of many writers shows that there is always less uric acid in the formed elements of the blood than in the plasma. Wu (1922) found 3.92 mg per cent uric acid in the blood plasma and 1.93 mg per cent in the erythrocytes of healthy people. Folin and Svedberg (1930) found 2.3–3.2 mg per cent of this substance in human blood, 3.5–4.8 mg per cent in the plasma and in the erythrocytes 22 per cent of the concentration in the plasma. Kosyakova (1930) investigated the distribution of uric acid between the erythrocytes and the plasma in the blood of children. She found that there is 1–2 mg per cent less uric acid in the erythrocytes than in the plasma. These results are in good agreement with the results of the other workers cited and with the results of other writers whom she mentions in her work.

Folin, Berglund and Derick (1924) found that in the muscles of rabbits and dogs uric acid is present in greater amounts than in the blood plasma. Further, in experiments with cats, rabbits, dogs and goats they established that uric acid introduced into the blood accumulates in large amounts in the kidneys and in lesser amounts in the liver, while it apparently fails to penetrate the muscles at all. They supposed that the uric acid that forms in the muscles is emitted by them into the blood by secretion.

Recently, Christensen (1961) summed up the work on the action of certain hormones and their homologues on the permeability of cells for amino acids and uric acid. In his opinion, the hormones act on the mechanism of membrane transport, some stimulating its activity, others depressing it. For example,  $\beta$ -oestradioldisulphate facilitates the penetration of uric acid into human erythrocytes, acting as it were as a chemical mediator for this process.

#### 5. The Permeability of Cells for Fatty Acids.

According to Overton's lipoid theory the permeability of cells to a number of fatty acids should grow with increasing number of carbon atoms in the molecule—in parallel with their increasing solubility in fat-like substances (Overton, 1902a; Höber, 1945).

Green (1948, 1949) determined the speed of penetration into the erythrocytes of humans, cattle and *Mustelus canis* of a number of fatty acids (formic, acetic, propionic, butyric, valeric, caproic, heptylic and caprylic). The speed of their penetration into the cells was found from the speed with which 50 per cent haemolysis occurred in solutions of these substances and spectrophotometrically by the degree of dissociation of oxyhaemoglobin, which changes on the penetration into erythrocytes of an acid. In general it was found that with increasing molecular weight of the fatty acid its speed of penetration into the cells grew. However, when the number of carbon atoms in the molecule went above six, a constant lowering was again observed of

the speed of their penetration into the cell. The author remarks that the haemolytic and spectrophotometric methods do not yield homogeneous results. A difference is also observed depending on the erythrocytes belonging to one or other type of animal or human. Green came to the conclusion that the speed of penetration of fatty acids into a cell depends on their solubility in the lipoids and on the size of the molecules. Similar conclusions were reached by Love (1953), who used the haemolytic technique to study the permeability of the erythrocyte-like cells of *Phascolosoma gouldi* for the ammonium salts of a number of fatty acids.

### 6. Conclusions

1. Live cells are permeable for a very wide variety of amino acids, just as they are permeable for ascorbic acid, its dehydro forms, pyruvic, uric and fatty acids.
2. The ability of cells to accumulate amino acids within them varies for different amino acids. Exactly in the same way the different cells of the body differ in the intensity with which they absorb from the medium both individual amino acids and ascorbic and dehydroascorbic acid.
3. Normally, the concentration in the cells of the nitrogen of amino acids and polypeptides of ascorbic acid (except for erythrocytes) and of pyruvic acid (in the erythrocytes) is greater than in the plasma, while the concentration of uric acid, on the other hand, is less in the erythrocytes and greater in the other cells of the body than in the surrounding medium.
4. The concentrations of amino acids in cells can be both greater and smaller than in the medium, depending on their concentration in the latter. At small concentrations there is more in the cells, at high more in the medium.
5. The preponderance in the cells or in the medium of amino acids, and likewise of ascorbic acid, its dehydro form, pyruvic and uric acid, may be due to the same factors as the analogous phenomenon in the distribution of non-electrolytes: the preponderance of these acids in the medium is caused by their lowered solubility in the live matter as compared with their solubility in the aqueous medium; while their preponderance in the cell is due to their adsorptive and chemical binding by the colloids of the protoplasm.

## CHAPTER VII

### The Permeability of Cells for Vital Dyes

#### 1. *The Distribution of Vital Dyes between Unexcited and Undamaged Cells and the Medium*

The permeability of plant and animal cells for dyes has been the subject of a particularly large amount of work. Experiments with these materials which permit the visual tracing of diffusion processes and the subsequent fate of the dyes in various physiological states of the live object have yielded much valuable information on the laws of cell permeability.

In agreement with the views of Pfeffer, Overton, Höber and the other founders of the membrane concept, the majority of research workers consider that the penetration of these substances (just like others too) into the live cell is determined by their ability to pass through the protoplasmic membrane. According to Overton (1895–1900) basic vital dyes very quickly penetrate the cell and can accumulate there in considerable quantities, while acid dyes do not, apparently, have these properties. In Overton's view this phenomenon is connected with the fact that the former are soluble in lipoids and the latter are not. However, it was later shown both in plant (Ruhland, 1912 a and b; Rohde, 1917; Collander, 1921 and many others) and in animal cells (Walbach, 1931; Gellhorn, 1929, etc.) that live cells are penetrated by both acid and basic dyes, whether soluble or insoluble in lipoids.

In the opinion of Kinzel (1954) and many other workers, molecules of the basic vital dyes penetrate the vacuoles of plant cells in the undissociated form and are dissociated again in the vacuole.

For the understanding of the laws of the passage of substances into cells and their distribution between the cells and the medium, observations of the change of colouring by vital dyes of the protoplasm and nucleus in relation to their functional state are of the greatest significance. This problem has in the last twenty years been the subject of comprehensive investigation by Nasonov and his colleagues and by many other workers. The literature on the distribution of dyes in the cell is set out very fully in the books of Nasonov and Aleksandrov (1940) and Nasonov (1959 a).

The work of many writers has established that, on passing into the cell, vital dyes at the first instant colour the protoplasm very weakly and diffusely and often fail to colour the nucleus at all. This state of affairs lasts only a very short time (a few minutes in all). Soon the diffuse colouring of the proto-

plasm fades—the dye that has penetrated the cell begins to colour strongly some antecedent structures or inclusions (yoke nuclei, digestive vacuoles of protozoa, vacuoles of plant cells, etc.). However, the vital dyes often form new granules, which initially appear in the form of little droplets and then increase in size and finally merge. This process of the formation of new granules in the cell had been observed many years ago.

It was also noted that not all vital dyes can precipitate in cells in the form of granules. Accordingly a distinction is made between granular and diffuse vital dyes.

According to Nasonov and Aleksandrov (1940), the basis of the formation of granules of vital dyes is the same mechanism which, in the normal conditions of cellular activity, leads to the formation of granules of pigment, secretion, lipoid inclusions etc. (see Nasonov, 1959a).

Recently Rumyantsev (1959) in experiments with various epithelial cells of mice and frogs found that granules can be formed in cells which contain not only vital dyes but also many substances in the alkaloid series. Novocaine, cocaine, strychnine, morphine, quinine, quinidine and atebrin in certain amounts provoke the formation in the cytoplasm of colourless granules. These granules possess a number of properties similar to those of granules of vital dyes. In the case of quinine, quinidine and atebrin their presence in these granules was proved, which attests the permeability of cells for alkaloids.

Nasonov (1926, 1927) discovered that the formation of these new granules of vital dyes and other substances takes place in the region of Golgi's apparatus, which is directly related to this process.

Chlopin (1927) showed that the formation of new granules in the cell is connected with the deposition together with the dyes of the proteinaceous substances of the protoplasm. Chlopin's data were subsequently confirmed by Kryukova (1929), Nasonov (1930), Kedrovskii (1931a and b, 1937), Aleksandrov (1932) and Rumyantsev (1935).

In a series of papers Fel'dman (1948c, 1950, 1953) convincingly showed that the process of the formation of new granules of vital dyes in the protoplasm of animal cells is the process of the formation of droplets of a complex or autocomplex coacervate, just as happens in the vacuoles of plant cells on the introduction into them of vital dyes (Bungenberg de Jong and Bank, 1939a and b, 1940; Bungenberg de Jong and Kok, 1940; Bungenberg de Jong, Kok and Kreger, 1940; Bungenberg de Jong and Kreger, 1940; Guilliermond, 1941, and others).

Nasonov and Aleksandrov (1940) point out that the formation of granules in the protoplasm from the various substances that have penetrated the cell is a widespread biological reaction. The work of Politzer (1926) and Aleksandrov (1939b) established that the formation of new granules of vital dyes in the protoplasm is a protective reaction of the cell in answer to the introduction into it of an alien substance. The vital dye that penetrates the cell becomes less poisonous if it is separated off into granules. This is confirmed

by the fact that those vital dyes which cannot be separated off into granules and colour the protoplasm diffusely are more poisonous than those which are capable of formation into granules (Aleksandrov, 1939 b, Fel'dman, 1948 a and b; Braun and Fel'dman, 1949; Krasil'nikova, 1950).

The protoplasm in its unexcited and undamaged state is coloured very weakly, whereas, as a rule, the cytoplasm and nucleus are coloured considerably more weakly by acid than by basic dyes. In most cases the diffuse colouring of the protoplasm normally vanishes quickly, the dye is collected into granules, and the protoplasm frees itself of the dye (Nasonov and Aleksandrov, 1940; Nasonov, 1959 a).

In plant cells the formation of granules of dyes takes place in the vacuoles. The abundant literature on the problem is set out in the monographs of Guilliermond (1941) and Meisel' (1950), and also in the works of Strugger (1936) and Aleksandrov (1955).

The permeability of erythrocytes has been studied quantitatively for a great variety of dyes. Thus, Yurišic (1927) determined the absorption by bull erythrocytes previously washed in a 0·9 per cent solution of sodium chloride of a number of basic and acidic dyes. This writer found that the basic and acidic dyes that are soluble in lipoids (basic dyes: rhodamine B, brilliant cresyl blue, toluidine blue, neutral red, etc.; acidic dyes: tropeolin, orange, eosine, etc.) are distributed between the formed elements of the blood and the surrounding solution according to Henry's law. For all concentrations of these dyes in the medium their content in the erythrocytes was several times greater (from 1·5 to 2·2 times depending on the dye and independent of concentration). On the basis of these data the author came to the conclusion that erythrocytes should be considered entirely as a phase in relation to an aqueous solution of the dyes mentioned. Colloidal acidic dyes (fast scarlet) are absorbed by erythrocytes in agreement with the laws of adsorption, though there is one peculiar feature, namely, that with increasing temperature the absorption of these dyes by the erythrocytes also increases.

Bruch and Netter (1930) studied the distribution between erythrocytes and medium of basic (toluidine blue) and acidic (orange R) dyes and came to the same conclusions as the preceding author: these dyes quickly penetrate erythrocytes and are distributed between the cells and the medium as between two phases.

The assertion of Yurišic, Bruch and Netter that the distribution of dyes between erythrocytes and the surrounding medium obeys Henry's law is, to all appearances, mistaken. These authors worked with small concentrations of these substances. If erythrocytes adsorb dyes strongly, then in these conditions the adsorption isotherm will at first look like a straight line for a long way. By dealing only with the beginning of the adsorption isotherm they took it, evidently, as an example of Henry's law.

Woodhaus and Pickworth (1932) studied the penetration into washed sheep erythrocytes of 33 dyes. All these penetrate the cell at various speeds

and accumulate there; from the data obtained it is not possible to reach any conclusion about which dyes (acidic or basic, soluble in lipoids or insoluble) penetrate erythrocytes more quickly. For example, at one and the same time, the basic dye neutral red penetrates acidic blood cells in smaller quantities than fuchsin.

Gilbert and Blum (1942) made a spectrographic study of the distribution of bengal rose between washed rabbit erythrocytes and salt solution. They established that this dye, which is soluble in lipoids, penetrates erythrocytes fairly quickly (they assert, only into the membrane and not inside the erythrocyte) and its concentration reaches 72 per cent of the concentration in the medium. Complete equilibrium is reached after about 2·5 hr. The dependence of the concentration of the dye in the cell (as they assert, in the cell membrane) on its concentration in the medium is linear over a wide range. In the conclusion of this work they suggested that the dye is first quickly dissolved in the lipoids of the membrane and then chemically bound by the proteins in this membrane, never actually penetrating inside the erythrocyte.

Maurer (1938) and Yermilova (1959) studied the permeability of frog muscle fibres for phenol red. They found that the concentration of phenol red in conditions of diffusion equilibrium, which is reached after 0·5–2 hr, is 3–4 times less than in the surrounding solution. Dead muscles absorb considerably greater amounts of this dye. The same results were also obtained by the present author (Troshin, 1951d).

Commoner (1938) studied the accumulation of neutral red by unfertilised *Chaetopterus* eggs and came to the conclusion that the accumulation of dye by these cells is caused by adsorptional binding by the cell proteins.

## *2. The Distribution of Vital Dyes on Stimulation or Damage of the Cells*

The distribution of dyes between cell and medium described above is characteristic only for cells in the resting state. When the cells are physiologically active or when they are subject to the action of a number of agents, differing widely in physical or chemical nature, the distribution of the dye may be sharply changed and the ability of the protoplasm to form granules disappears, also the cytoplasm and nucleus become diffusely coloured, the nucleus earlier than the cytoplasm. Vital dye-indicators show that this is accompanied by a displacement of the intracellular pH towards the acid side. This type of colouring of an altered live substance is perfectly reversible when the cells are placed in normal conditions, provided that the action of the external agent has not gone too far: the protoplasm and nucleus take on their normal colour, granules of dye appear in the protoplasm which may be coloured by granular dyes and the concentration of hydrogen ions falls.

The change in the character of the vital colouring of live cells under the

action of a wide variety of agents was studied in detail in the laboratory of Nasonov and then in other laboratories.

Beside the change in the tinting properties of the protoplasm under the action of agents on the cell, the following phenomena are also observed:

(1) reduction in the dispersion of the colloids of the cytoplasm and nucleus, accompanied sometimes by their gelatinisation;

(2) increase in the viscosity of the cytoplasm, which is in some cases preceded by its reduction;

(3) increase in the concentration of hydrogen ions in the cell.

This complex of changes in the protoplasm under the action of various agents in the reversible phase was called paranecrosis by Nasonov and Aleksandrov (1934). This phase of substantial changes in the live matter coincides with the phase of reversible suppression of the cell functions (narcosis) in the parabiotic process (Vvedenskii, 1901).

A large amount of information has by now been accumulated which permits to be considered paranecrosis as a process which proceeds by phases, exactly as happens in parabiosis (Nasonov and Suzdal'skaya, 1948; Romanov, 1948 a, 1949 a; Ushakov, 1949, 1951, 1954; Zarakovskii and Levin, 1950, 1953; Nasonov, 1951; Aleksandrov, 1951; Nasonov, 1959 a, b, etc.).

Certain data relating to this problem will be set out below.

*The effect of various irritants on the distribution of vital dyes.* Nasonov (1932 a) observed the paranecrotic type of colouring by neutral red of the intestinal epithelium of the frog under the action of elevated temperature (36–38°) for 20–60 min. Similar results were later obtained by Braun and Ivanov (1933, 1938) in experiments with frog muscles, by Aizenberg (1934) in experiments with the epithelial cells of frog's web in the intact animal when dyed with neutral red. Meshcherskaya (1935) observed a similar picture of the distribution of neutral red in the cells of various insect tissues when subjected for 2 hr to a temperature of 39–40°. Vishnevskii and Lavrent'yev (1939 a, b) described the greater colourability by methylene blue of both protoplasm and nucleus of nerve cells of warm-blooded animals when subjected to high and low temperatures, also under the action of acids, bases and mechanical trauma. According to the observations of Levin (1949 a, 1951 a), the protoplasm and nucleus of the epithelium of the posterior elastic lamina of the cornea of white mice and rats are diffusely coloured by neutral red when the tissue is subjected to a temperature of 46° for 10–30 min.

In Nasonov's laboratory, Braun and Ivanov (1933) developed a quantitative method of calculating the absorption of dyes by tissues, which significantly extended the application of the method of vital dying for the determination of the nature and functional state of live protoplasm. They found that, if the colour of the one leg muscle when coloured at 19° is taken as 100 per cent, then the twin muscles coloured at 25° contain 33 per cent more neutral red, coloured at 31° 42 per cent more, coloured at 33° 68 per cent more than the control. In view of the fact that muscle fibres are partic-

ularly weak in the formation of granules of neutral red, this elevation of the colouring of the experimental muscles must be ascribed almost entirely to the increase in the sorptional properties of the cytoplasm and nucleus.

The increased affinity of frog muscle tissue to neutral red under the action of elevated temperature was also observed by Butkevich-Troshina (1948).

Increase in the sorption of neutral red by rabbit muscles was observed by Chagovets (1938) at elevated temperatures and in muscle fatigue.

Krasil'nikova (1954) observed the suppression of the precipitation of granules of neutral red and novomethylene blue in the kidney epithelium of mice under the action of elevated temperature, hypotony, and strychnine nitrate. In a certain zone these agents caused strong diffuse colouring of the epithelium of the ducts by basic and acidic dyes.

As was shown by Grayevskii (1946, 1948), Grayevskii and Strelin (1947) and Grayevskii and Medvedeva (1948), strong refrigeration leading to the formation of ice in the cells (yeast cells, frog muscles) also leads to increased affinity of the cytoplasm to dyes, but on cooling to the temperature of liquid air without the formation of ice in the cells (vitrification) no such phenomenon was observed.

According to the observations of Paltauf (1928), raising the temperature to 35° leads to considerably increased colouring of the protoplasm and nucleus of the bulb cells of various plants by acidic dyes (eosine and erythrosine), it being important to note that, according to her observations, the nucleus is more strongly coloured than the protoplasm, while the cell juice is not coloured at all.

In Ushakov's laboratory a detailed study has been made of the temperature threshold of the onset of the paranecrotic type of colouring by vital dyes of muscle, nerve, epithelial and other cells of various types of cold-blooded animals. It was established that this threshold varies for cells of different kinds. It is higher for the heat-loving types than for the cold-loving (Ushakov, 1955, 1956, 1959 a, b, c, 1960 a, b; Kusakina, 1959 a, Shlyakhter, 1960). It was also shown that these differences are caused by the properties of the cell proteins (Ushakov, 1955, 1956, 1959 a, b, c, 1960 a, b; Ushakov and Kusakina, 1960; Braun, Nesvetayeva and Fizhenko, 1959).

According to the data of Kryukova (1938), the sarcoplasm and nucleus of frog muscle fibres are strongly coloured by neutral red when cut. The damage caused by the cutting spreads along the fibre, the zone of increased colouring of the sarcoplasm and nuclei spreading as the zone of damage widens. This phenomenon was then studied in detail by Nasonov and Rozenthal' (1947), Rayevskaya (1948), S. Aleksandrov (1948 a, b, 1949), Gramenitskii (1948), etc.

The experiments of Bank (1933) with the cells of onion shells are of great interest. After the cells have been 2 hr in a weak solution of the basic dye, nile blue sulphate, only the juice of the vacuoles is strongly coloured, the nuclei and cytoplasm remaining uncoloured. If a light pressure is put upon

such cells, an immediate redistribution of the dye takes place: it moves out of the vacuoles into the protoplasm and colours the nucleus intensely.

Intense colouring of the nucleus by neutral red in the case of mechanical action on the cells of the fungus *Basidiobolus* was observed by Becker and Skupienski (1935).

Lepeschkin (1925) described diffuse colouring of the protoplasm and nucleus by neutral red and methylene blue in the case of mechanical action on protozoa.

Reversible increase in the sorptional properties of the cytoplasm and nucleus and all the other signs of paranecrosis are also caused by dilution of the saline medium in which the cells normally live (blood plasma, Ringer's solution or sea water). This was observed by Kamnev (1934a, b) in the cells of the intestinal epithelium of the frog on the introduction into the cavity of the frog's body of distilled water. The same writer (1936) described a similar picture of the change in the tinting properties of the cells of many frog organs and tissues when isolated.

Kamnev's results were later confirmed by Fedorov (1934) for the action of hypotony on the nerve cells of frog's heart, by Meshcherskaya (1935) for the action of the same factor on the cells of various insect tissues and by Smoilovskaya (1938) in the cells of live sections of the liver, kidneys and lungs of rats, rabbits and guinea pigs. It is interesting that in Smoilovskaya's experiments when the Ringer's solution was diluted more than twice, causing diffuse colouring of the nucleus and cytoplasm and suppression of granule formation, an increased amount of phosphates was given up by the live sections of these organs. Kamnev's results were also confirmed by Ries (1937) and Grossfeld (1937a, b, 1938) in the cells of various tissues of amphibians.

Braun and Ivanov (1933) made a quantitative determination of the increase in the binding of dye by frog muscles on immersion into Ringer's solution diluted to various degrees. They obtained a maximum increase in colouring of the muscles of 100 per cent by comparison with the colouring of the control muscles. Using the same subject but colouring with a different basic dye—dahlia violet, whose adsorption does not depend on the concentration of salts in the medium (in contrast to many other acidic and basic dyes), Nasonov and Aleksandrov (1937) obtained a maximum increase in colouring of the experimental muscles over the control of 50 per cent.

Colouring of the paranecrotic type of cells of the surface epithelium of the cornea of the eyes of white mice was observed by Levin (1949a) on immersion of the tissue for 12–30 min in Ringer's solution diluted five times with distilled water. The same phenomenon was described by Gavrilova (1948) after experiments with frog skeletal muscles.

Cessation of granule formation and diffuse colouring of the nucleus and cytoplasm were observed by Yel'tsina (1946) in sections of malignant tumours and liver cells under the effects of hypotonic saline and by Aizenberg-Terent'yeva (1950, 1951) in experiments with lymphocytes of the normal blood

of humans, dogs and rabbits, and also in experiments with the cells of the blood-making organs of rats under the action of hypotonicity, ether and acetic acid.

Bank (1936, 1938) observed the vital dyeing of the nucleus by acidic and basic dyes in the cells of onion shells under the action of 1 M sodium chloride solution and rhodanides. At the same time, structures appeared in the nuclei. According to Paltauf (1928), sodium, potassium, magnesium and calcium nitrates in concentrations of 0·25–0·5 per cent cause diffuse colouring of the cell nuclei of bulbs of various plants by eosine.

Lepeschkin (1925) and other writers have described the suppression of the formation of granules of vital dye and the diffuse colouring of the protoplasm of protozoa under the effects of hypotonicity.

Becker and Beckerowa (1934) passed a direct electric current through onion epidermis and at the same time coloured the cells with neutral red. Almost immediately after the current was switched on intense colouring was observed of the nucleus, nucleolus and nuclear envelope. This phenomenon was fully reversible.

Changes in the colouring properties of live matter under the effects of direct electric current were studied in detail in a series of papers by Kamnev (1939a, b, c, 1941, 1947, 1948, 1949) on the striated muscle structure of frogs and on the cells of the cornea of the eyes of various animals (frog, rabbit, cattle etc.). The passage of electric current through these tissues causes a whole complex of paraneurotic changes.

The distribution of vital dyes in cells is similarly affected by X-rays and ultra-violet light and also by visible light when this is thrown on cells that have previously been dyed with dyes capable of the photodynamic effect (Nasonov and Aleksandrov, 1940; Lozina-Lozinskii, 1955)\*.

Nasonov and Ravdonik (1947) dyed isolated frog sartorius muscles with neutral red and cyanol when the muscles were subjected to sound of various frequencies (from 200–10,000 c/s) and the same intensity equal to 95 dB. It turned out that the muscles, when subjected to the action of sound absorb greater quantities of acidic and basic dyes than the control muscles, the maximum colouring occurring in the frequency zone from 2500–3000 c/s, and the colouring falling off towards greater and lesser frequencies and approaching the colour of the control muscles (Fig. 68). The writers showed that sound of such intensity and such frequencies had no effect on the colouring of muscles fixed in alcohol. Further, it was found that in the same frequency zone in which maximum colouring occurs, the muscles respond to this sound irritant by prolonged contraction. These results were later confirmed on the same subject by Ravdonik (1949). Furthermore, this writer showed that the intensity of the colouring of frog muscles by neutral red depends on the intensity of the sound at one and the same frequency of oscillation.

\* On the effect of radiant energy on the permeability of cells and tissues see the references cited in Aleksandrov (1934).

Nasonov and Rozental' (1950) in the same frequency zone obtained maximum binding of phenol red (an acid dye) by the cells of the epithelium of the kidney tubules of frogs, while Nasonov and Ravidonik (1950) obtained the same results for the colouring by neutral red of the cells of rabbit ganglia.

Romanov (1954) discovered that 4–6 hr after rabbits have been subjected to an explosive sound of 200 dB power, isolated sacral ganglia and upper cervical sympathetic ganglia show an increase in the absorption of neutral red of about 20 per cent by comparison with the control. At the same time

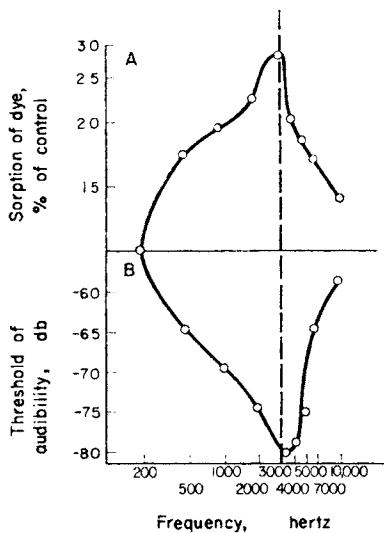


FIG. 68. The change in the sorptional properties of frog muscles (A) and in the threshold of audibility of the human ear (B) on the action of sounds of varying frequency (from Nasonov and Ravidonik, 1947). The sorption of the dye and the frequency are given on logarithmic scales. In reading 1 bar is taken as 0 db.

the cells of the cornea of the eyes of the experimental animals are 18·7 per cent more weakly coloured than the control. After 20–24 hr the sorption of neutral red by the cervical sympathetic ganglia and the cells of the cornea of the eyes of the experimental rabbits approach the control, while the sacral ganglia become 14·1 per cent lower than the control.

Subsequently a number of workers confirmed the results which have been described above and also obtained new information on the effect of acoustical irritation on the amount of sorption of vital dyes by nerve and other cells of mice, rabbits, guinea pigs and other animals (Zhirmunskii, 1957; Vinikov and Titova, 1957; Timofeyeva, 1960).

The action of narcotic substances on various animal and plant cells was observed to present the same picture of paraneurotic changes in the cyto-

plasm and nucleus as was observed by Nasonov and his colleagues when cells were subjected to elevated temperatures, mechanical damage and other agents.

The effect of narcotics on the vital colouring of protozoa and the cells of various tissues of many representatives of the animal kingdom was studied in detail in Nasonov's laboratory by Makarov (1934a, 1935a, 1935b, 1936, 1938).

Nasonov and Aleksandrov (1937), using the method developed by Braun and Ivanov (1933), made a quantitative study of the increased ability of frog gastrocnemii to adsorb acidic and basic dyes after the action on them of a number of narcotics (methyl, ethyl, propyl and butyl alcohol, acetone, ether, urethane, chloral hydrate). All these substances after 1·5 hr action cause increased absorption of the dyes by the muscles, the binding of the dye by the muscles increasing with increasing concentration of the narcotic. It is important to notice that the increased colourability of the muscles which have been treated with narcotics begins even when subnarcotic doses are given, before the excitability of the muscles is reduced. Similar effects are produced not only by typical narcotics, but also by such substances as saponin, urea, glucose, sucrose, glycerol, and, according to Sutulov (1937a, b, c, 1938, 1940) by acetylcholine, optochine, caffeine and hydrogen sulphide. Rayevskaya and Troshin (1937) found the same law when they investigated the effect of ethyl alcohol and quinine on frog spermatozooids.

Vol'fenzon (1949) observed colouring of the paranecrotic type in the cellular elements of various rabbit tissues when anaesthetised. The same was observed by Levin (1949b) studying the effect of ether and ammonia vapour on the cells of the hypoderma of fly larvae. Suppression of granule formation and colouring of the nucleus with neutral red were achieved by Karasik and Lutovinova (1948) by the action on frog erythrocytes of synthetic (racemic) and basilic (dextrorotatory) camphor.

Colouring of the paranecrotic type by neutral red of the surface epithelium of isolated cornea of white mice and rats was observed by Levin (1951a, b) on treatment with strychnine nitrate.

On the simultaneous introduction into the blood or body cavity of frogs and mice of a vital dye and novocaine (Makhover, 1959) or a lead nitrate solution (Gramenitskii, 1958; Gramenitskii and Makhover, 1958) there was similarly found to be suppression of granule formation in the various cells of the body and diffuse colouring of these cells. A similar picture of the change in colouring of nerve and other cells is observed when isolated frog and mouse organs are subjected to the action of lead nitrate (Shumova, 1958), adiphenin (Brovkovich and Zapletalova, 1958) and other poisons.

Il'yinskaya (1957) showed that the cells of the thoracic ganglion and the middle section of the intestine of flies begin to adsorb phenol red and neutral red strongly already in the first hours after the beginning of contact by the insects with DDT. It was shown that the flies die of the insecticide through

destruction of the nervous system, which is admirably shown by the nature of the change in the colouring of the nerve cells by vital dyes.

Kirillova and Popova (1959) found that the cells of many organs and the proteins of the serum of old rats bind more vital dyes than those of young rats. According to the results of Genni (1947) the action of acetylcholine on isolated rabbit muscles causes increased adsorption by them of neutral red, the muscles of new-born rabbits absorbing more of the dye than those of full-grown animals. This is very well illustrated by the data shown in Fig. 69.

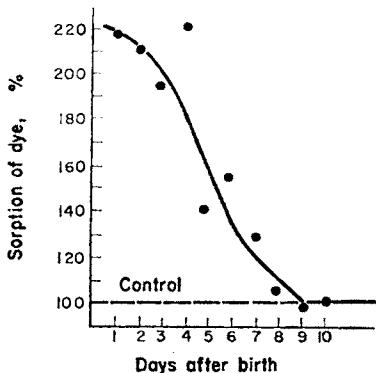


FIG. 69. The effect of acetylcholine on the sorption of neutral red by the muscles of newborn rabbits (in per cent of the mean values adsorbed under the same conditions by the muscles of fully-grown rabbits) (from Genni, 1947).

Of great interest are the observations of Meisel' (1938a) on the accumulation of Janus green in yeast cells. Normally Janus green in weak concentrations colours chondriosomes during their lifetime. On the passage of ether and chloroform vapour through the chamber in which the yeast cells are placed a rapid redistribution of the dye takes place: the Janus green leaves the chondriosomes and colours the cytoplasm. After removal of the narcosis the dye passes out of the cytoplasm and again colours the chondriosomes.

Genni (1947, see also Ginetsinskii, 1947) and Kiro (1948) observed an increase in the sorptional properties of frog muscles for vital dyes under the action of acetylcholine. The same result was obtained by Topchiyeva (1948) in the case of the nerve cells of frog's hearts for Gramenitskii's compound.

This termination of the ability of the cytoplasm to form granules of vital dyes and the increase in the diffuse colouring of the protoplasm and nucleus also occur at certain periods of the conservation of tissues in cold conditions (Muchnik, 1949, 1950; Grigor'yeva, 1950; Troshina, 1953), in wound complications (Makarov, 1945), in fatty degeneration of the sebaceous glands (Aleksandrov and Kryukova, 1949), in dystrophic change of the liver of rats and mice (Petrov, 1957) and so on. Belovintseva (1952) found that after

removal of the suprarenal glands frog skeletal muscles absorb neutral red on an average 22 per cent more than the muscles of the control animals.

Reversible increase in the affinity of the cytoplasm and nucleus of live cells and other signs of paranecrosis were observed by Nasonov (1932a) in cells of the intestinal epithelium of frogs on the introduction into the body cavity of a 3 per cent solution of boric acid and into the intestine of neutral red. Similar findings were obtained by Fedorov (1934) in the sympathetic nerve cells of frog hearts under the action of weak solutions of acetic acid coloured by basic dyes; by Meshcherskaya (1935) in the cells of the somatic tissues of insects on the introduction under the chitin of boric acid and neutral red; by Ries (1937) in the epithelial cells of the tail of the axolotl in colouring with neutral red after subjection to weak solutions of acetic acid; by Levin (1949a, 1951a) in the surface epithelium of isolated corneas of white rats and mice when these were placed for 20–25 min in hydrochloric acid solution of  $\frac{1}{1100}$  –  $\frac{1}{1400}$  N; and by Semenov (1934) in experiments with protozoa in which the pH was displaced towards the acid side.

Thus, the action of hydrogen ions on live cells produces an increase in the sorptional properties of live matter for basic dyes. This indicates deep reactive changes in the protein substrate of the cells, because on increase in the concentration of hydrogen ions the adsorption by dead proteins of basic dyes falls, whereas it rises for acidic dyes. It is only at the iso-electric point that dead proteins absorb both basic and acidic dyes in equal amounts (Pischinger, 1926, 1927). On this basis we should expect that the effect of acid on the protoplasm and nucleus would be to lower their colorability by basic dyes.

The action of a base on live cells is to cause a strong increase in the absorption of basic dyes by live matter. This was proved by Braun and Ivanov (1933) who used a quantitative method to study the striated muscles of frogs, introducing sodium carbonate solution or Ringer's solution (pH = 11·0) into the lymphatic sac and subsequently colouring the muscles with neutral red. A considerable increase in the colourability of frog muscles by methylene blue was observed by Nasonov and Aleksandrov (1934) on the addition of ammonia to the medium to make it basic, and by Vishnevskii and Lavrent'yev (1939a) in the case of nerve cells when a base was applied to the serous membrane of rabbit intestine.

Kuwada and Sakamura (1927) described the staining of the live matter of plant cells by basic dyes under the action of acids. Colouring of the cell nuclei of various plants by acid dyes (eosine and erythrosine) was discovered by Gicklhorn (1927, 1930).

To understand the laws of the absorption of cations and anions by cells it is important to study the work which was done on the distribution of dyes between plant cells and the medium in conditions of variable pH. It was established by this work that it depends on the pH of the medium whether it is the cell wall or the protoplasm or the cell juice that is stained.

Experiments of this kind were performed by Strugger (1936) who placed the cells of the leaves of onion bulbs in weak solutions of neutral red, prepared in buffer mixtures of various pH (Table 45). At very high concentrations of hydrogen ions in the medium ( $\text{pH} = 2\text{--}3$ ) the wall is not stained at all; further, in these conditions a wall which has previously been stained loses its colour. When placed in a solution of neutral red made up with tap water of  $\text{pH} = 7\cdot 5$ , these cells showed intense staining of the vacuole and a colourless wall. If such cells were left for 15–20 min in the preparation under a cover glass (to which oxygen has poor access), then the dye was

TABLE 45. THE COLOURING OF THE WALL AND VACUOLE OF THE CELLS OF THE SHELLS OF THE ONION BY NEUTRAL RED AT VARIOUS pH (from Strugger, 1936)

pH of medium	Wall	Vacuole
5·97	CC	N
6·07	CC	N
6·66	CC	C
7·18	C	CC
7·28	N	CC
7·84	N	CC

Legend: Coloured (C), not coloured (N).

observed to move from the vacuole to the cytoplasm and thence to the wall. After some time the vacuole and protoplasm became completely colourless, while the wall was intensely stained, as when the cells were stained in conditions of low pH (see Table 45).

According to the results published by Guilliermond (1941) and Meisel' (1950), it follows that the majority of basic dyes in normal conditions accumulate predominantly in the vacuoles of plant cells (neutral red, toluidine blue, nile blue, cresyl blue, etc.). Neutral red on passing into the vacuole through the thickness of the protoplasm is partially held there, but in such small quantities that the staining is not visible by normal microscopy. Fluorochromes begin to accumulate in the vacuole only after they are adsorbed by the protoplasm. An increase in the sorptional properties of the protoplasm leads to a reduction in the amount of dye in the vacuole. "Thus", writes Meisel' (1950, p. 40), "we can no longer entertain the idea that substances which accumulate in vacuoles pass into them freely, as it were filtering through the protoplasm".

Vital dyes can accumulate in considerable amounts in the vacuoles of yeast cells (about 3 g of neutral red per 100 g of pressed yeast). The accumulation of the dye in the vacuole depends on the amount of proteins, nucleo- and lipo-proteids, metachromatin and other substances present. Changing the pH of the medium permits repeated observation of the displacement of neutral

red from the vacuole to the wall in acid media and the reverse transition from the wall to the vacuole in basic media. Meisel' (1950) gives the following values of pH below which the following dyes are not taken up by plant cells: for nile blue 2·4, for cresyl blue 5·4–5·6, for neutral red 5·6. The pH at which staining of the vacuoles begins lies between 5·5 and 7·0. Staining of the vacuoles of different kinds of yeast by one and the same dye begins at different values of pH. It is interesting that methyl blue is taken up by the cytoplasm only at pH = 9·0–11·0.

The absorption by plant cells of acid dyes is also strongly dependent on the acidity of the medium. This was well shown by Strugger (1938). Using the fluorescent microscope, he studied the accumulation of fluorescein and other fluorescent substances in the leaf cells of water thyme and in the cells of the leaves of onion bulbs. These subjects were immersed in solutions of fluorescein, made up in phosphate buffer mixtures of various pH (from 4·5 to 11·0): the distribution of this substance in the cells after 40 min was investigated. In old water thyme leaves fluorescein accumulated only in the walls, while in the young cells the wall was very weakly stained but the protoplasm and nucleus were very strongly stained right up to pH = 8. Increasing the concentration of hydrogen ions in the medium leads to increased staining of the nucleus and cytoplasm.

Sabinin (1940) suggested an electrophysiological mechanism of the accumulation of cations and anions in plant cells. His idea was that the decisive factor in the process of the absorption of anions and cations by cells is exchange electro-adsorption and desorption. Since the principal adsorbents in the cell are the proteins, i.e., amphoteric substances, this process (adsorption and desorption) should depend on the pH value. Further, other cations and anions should also affect it. This theory is supported by the following facts.

Czaja (1936) placed bundles of spirogyra threads in 0·01 per cent solution of toluidine blue. In 4 min all colour had vanished completely from the solution, while the cell walls were strongly stained. However, there remained in the solution up to 91 per cent chlorine (the anion of the dye). Thus, the absorption of the dye cation proceeded in exchange for some other cations belonging to the cell wall. According to Sabinin and Kolosov (1935), when methylene blue is absorbed by the root system of wheat, potassium, calcium and other cations pass into the solution; this indicated that the absorption of dyes by cells is a polar exchange adsorption process. This is also supported by the fact that cell walls that have been stained with basic dyes lose their colour when treated with mineral cations (Brauner, 1933; Borris, 1937a, etc.). The rapid and intense staining of spirogyra cell walls by methylene blue is prevented by the solutions: 0·1 N potassium chloride, 0·04 N calcium chloride and 0·0008 N aluminium chloride (Brauner, 1933).

According to Sabinin, a shift in the pH in a cell due to the course of

metabolism or a change in the acidity of the medium changes the adsorptional properties in the first place of the cell proteins. Because the different parts of a cell can consist of different proteinaceous substances whose isoelectric point occurs at different pH values, the change in the sorptional properties of different parts of a cell is not uniform, so that dyes, like other ions, are first absorbed now by the wall, now by the protoplasm, now by the nucleus. This is also the reason, probably, for the redistribution of dyes between different parts of a cell that takes place on a change in the pH of the medium. Of course, any change in the acid or basic properties of the cell proteins caused by any factor should also lead to a corresponding change in the distribution of dyes within the cell, such as takes place on a change in the acidity of the medium.

*The distribution of vital dyes between excited cells and the medium.* In the course of the last 5–6 years a series of papers emanating from the laboratory of Nasonov and other centres has established that a wide variety of cells in the excited state, caused by adequate irritation and the most varied inadequate irritations, absorb acid and basic dyes in considerably greater quantities than resting cells in the same time.

It is well known that the contracture of muscles, which can be caused by the action of any irritant, is classified in Vvedenskii's theory of parabiosis (Vvedenskii, 1901) as a persistent local non-oscillatory excitation.

It is now established that, as the power of the irritant grows from a certain threshold level, the speed of onset of refractility (narcosis) begins to grow quickly. From this level there also begins rapid growth in the extent of the contraction, electronegativity and absorption of dyes. The staining of the muscles in the contracted state exceeds the staining of the control (resting) muscles by 30–180 per cent depending on the intensity of the irritation. It is characteristic that the increase in colorability, even though slight, precedes the onset of the contractions.

The whole of this complex of functional and substantial shift is observed in frog sartorius muscles when these are treated with isoamyl alcohol (Nasonov, 1948), mercuric chloride (Nasonov, 1949 b), high temperatures (Butkevich-Troshina, 1948), ethyl alcohol, ether, hydrochloric acid, potassium and sodium chloride (Nasonov and Suzdal'skaya, 1948), cations and anions (Suzdal'skaya, 1948, 1952), calcium, magnesium and barium chloride (Rozental', 1948), hypotony (Gavrilova, 1948), quinine hydrochloride (Nasonov and Rozental', 1948), chlral hydrate (Zelenkova, 1949), audible sounds of certain frequencies and intensities (Nasonov and Ravdonik, 1947; Ravdonik, 1949), photodynamic dyes activated by visible light (Lozina-Lozinskii, 1955), and high hydrostatic pressure (Golovina, 1955 b, 1958 a). Il'yinskaya and Ushakov (1952) described this phenomenon in the retractor muscles of *Phascolosoma margaritaceum* on an increase in the concentration of sodium chloride in the sea water in which the muscles of this animal had been placed. A similar picture was obtained in experiments on the same subject by Lopatina, Usha-

kov and Shapiro (1953): they used ethyl alcohol, formaldehyde and elevated temperature.

In the normal course of excitation muscle and nerve fibres and also nerve cells absorb greater amounts of a dye than the same elements in the resting state. Thus Kiro (1948) showed that tetanic shortening of frog sartorius muscle, caused by induced current as the irritant, increases the binding of neutral red by 21·9 per cent, while the action on the rectus abdominus

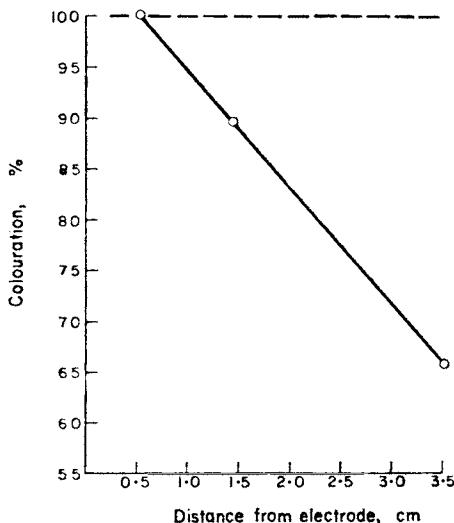


FIG. 70. The reduction in the binding of neutral red by the nerve of an edentate with increasing distance from the irritating electrolytes. The maximal absorption by part of the nerve of vital red is taken as 100 per cent (from Golovina, 1949).

of acetylcholine (tonic shortening) causes a 51·4 per cent increase in the binding of the same dye. Similar results were obtained by Vereshchagin (1949) stimulating tonic and non-tonic frog muscles by the nerve and by Shapiro (1960) in the case of stimulation of frog sartorius muscle.

Golovina (1949, 1955a) found in the case of the nerve of an edentate that the nerve impulse running along the axon causes a considerable increase in the sorptional properties of the conductor in relation to acid and basic dyes. Further, she established the decrement in staining corresponding to the decrement in the excitatory waves spreading along the nerve. These interesting results of Golovina's are shown in Fig. 70.

Later, Ushakov (1950) in the case of the nerves of the walking extremities of the crab *Hyas araneus* also found that excitatory waves running along the nerve which were caused by a Faradic current with an adequate frequency of irritation of 88–117 c/s, causes 11·4–15·5 per cent increase in the sorption of neutral red (Fig. 71).

Nasonov and Suzdal'skaya (1957) excited isolated sciatic nerves of winter frogs with sinusoidal current of various frequencies and found increased sorption of neutral red by the nerve with a maximum in the region of 100 c/s (Fig. 72). Lev (1959) established the dependence of the amount of absorption of neutral red by frog spinal ganglia on frequency (50–1000 c/s) and power of excitation. At a frequency of 100 c/s the experimental ganglia were stained

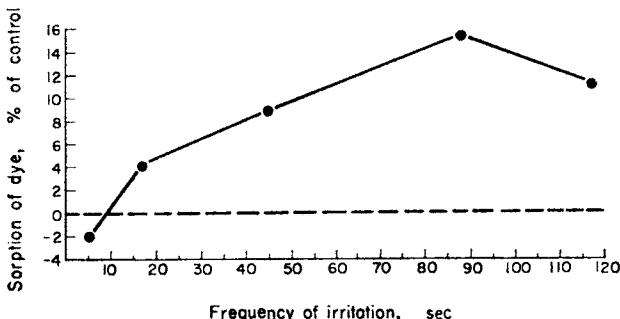


FIG. 71. The sorption of neutral red by crab nerve on irritation by induction currents of varying frequency (from Ushakov, 1950).

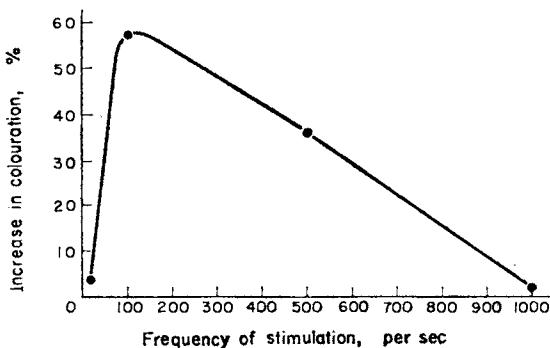


FIG. 72. The increase in the sorption of neutral red by the sciatic nerve of the frog on irritation by a sinusoidal current of varying frequency (after Nasonov and Suzdal'skaya, 1957).

less than the control and at a frequency of 500 c/s more. In another study on the irritation of the sciatic nerve by rectangular impulses of 0.8 msecs duration and 100 c/s frequency it was found in one case that there was an increase in sorption by the spinal ganglia and in another a decrease, depending on the initial functional state of the preparation (Lev and Rozental', 1958). However, under the same conditions rat spinal ganglia respond to excitation only by increased sorption of dye (Rozental', 1958).

When nerve cells are excited by nervous impulses the same general picture is observed of the change in distribution of vital dyes as is seen when cells

are subjected to the direct action of various irritants. If the excitation through the nerves is prolonged or above the threshold level the adsorption of dyes by the nerve cells grows sharply, the cytoplasm and nucleus being stained diffusely. Short-term or weak (sub-threshold level) excitations of a nerve by an

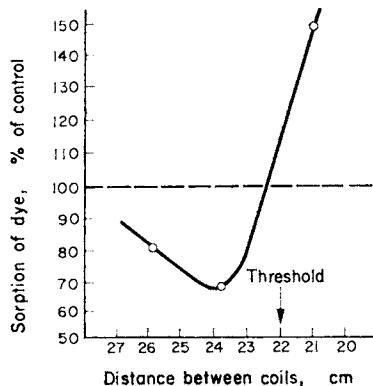


FIG. 73. The binding of neutral red (out of a 0·1 per cent solution) by the upper cervical sympathetic ganglia of a cat during a 30 min irritation by an induction current of 25 c/s frequency (from Zarakovskii and Levin, 1953).

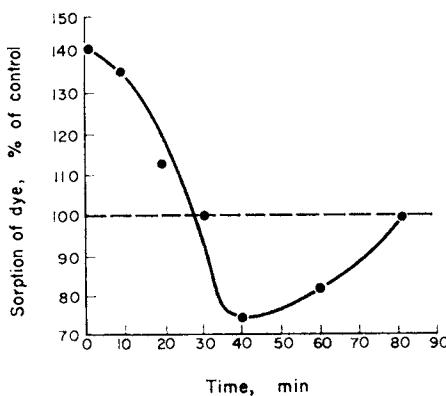


FIG. 74. The colouring of the spinal ganglia of liver rabbits by a 0·1 per cent solution of neutral red at various intervals after the cessation of irritation (from Romanov, 1948a).

induced current cause a reduction in the adsorption of dye by the protoplasm (Zarakovskii and Levin, 1950, 1953; see Fig. 73).

An increase in the binding of vital dyes was observed by Romanov (1946, 1948a, 1949a) when the nervous appendices of rabbit spinal cord ganglia were excited by induced currents of various duration and also after traumatic shock. Similar changes in the brain cells of frogs and mice are observed

when the sciatic nerve and the stomach receptors are excited (Romanov, 1957 a, b). When the cells were restored to their normal state, he observed a phase of lowered sorption of vital dyes (see Fig. 74). A similar phenomenon is described by Smitten (1948).

Biphasic change in *in vivo* colouring (reduction and subsequent increase in the binding of dyes) was also observed by Ushakov (1951) when he studied the effect on frog muscles of measured doses of ethyl alcohol and chloral

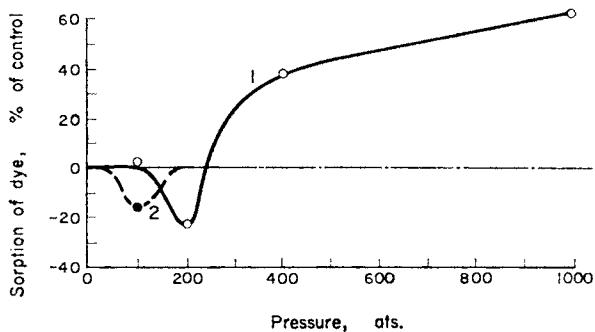


FIG. 75. The binding of neutral red by frog sartorius muscle during the action on it of hydrostatic pressure (from Golovina, 1955).

1—winter frogs; 2—spring frogs.

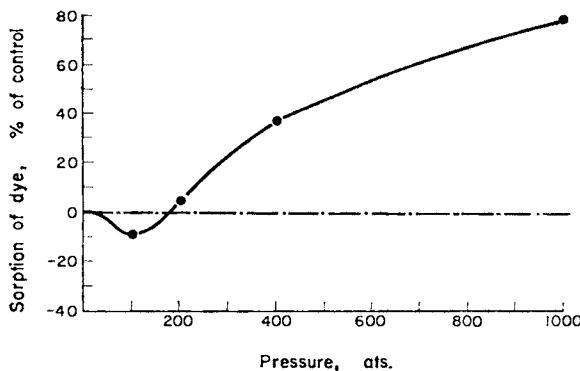


FIG. 76. The binding of phenyl red by frog sartorius muscle during the action on it of hydrostatic pressure (from Golovina, 1955).

hydrate, by Golovina (1955b, 1959) and Suzdal'skaya (1960) when hydrostatic pressures of various values were applied (see Figs. 75 and 76) and by Suzdal'skaya (1955) when the same effect was tried in the case of the digestive glands of mice. High hydrostatic pressure on isolated mouse brain has the effect only of strengthening the sorption of acid and basic dyes (Golovina, 1958b).

Biphasic change in the sorptional properties of the cells of various organs in relation to vital dyes was observed by Yakovlev (1958a, b, c, d) on the development in guinea pigs of experimental tuberculosis. The same effect was noted by Kusakina (1957, 1959b) in experiments with isolated frog muscles to determine the effect of prolonged action on the muscle of 0·05 per cent caffeine solution, 0·25 per cent chloral hydrate solution and pressure. In these experiments it was shown that the phase of lowered sorption coincides with increased transparency of the muscle, and the phase of increased sorption with lowered transparency (clouding of the muscle; see Levin, 1960).

When isolated frog muscles and corneas, and also mouse brains, are kept for lengthy periods at temperatures close to zero, periodic increase and decrease in the absorption of vital dyes by the cells is observed. The excitability of the muscles varies periodically. In long periods of preservation very strong (pathological) granule formation is observed in the muscles (Troshin, 1956, 1957). When isolated frog muscles are kept in Ringer's solution at room temperature there is a similar oscillatory change in their sorptional properties for vital dyes, their excitability and the magnitude of the resting current (Vinogradova, 1959, 1961). Zhirmunskii (1958) found the same change in the properties of rat skeletal muscle at various times after denervation of the muscles.

The work of Levin (1952) and Zarakovskii (1952) is of great interest. Levin showed that when the mechano-receptors of different parts of the stomach-intestinal tract of mice are excited, the sorptional properties of the nerve cells of the cerebral cortex are increased by 46·1-64·6 per cent for neutral red and by 23·5 per cent for phenol red. Similar results were obtained by Zarakovskii in experiments with frogs when neutral red was used to stain the skeletal muscles while the spindle-receptors were simultaneously excited.

Increase in the sorptional properties of the ends and the terminal ramifications of the motor nerve in frog sartorius muscles was found by Shapiro (1953) using methylene blue as stain when there was simultaneous excitation of the muscles with induction currents through the nerves. These results were later confirmed by Chetverikov (1953).

Zhinkin and Korsakova (1951a, b) in experiments with white rats showed that when part of the cornea is burned, there is a sharp change in the adjacent (undamaged) part in the mitotic activity of the cells and also in the absorption by them of neutral red. In the first 18 hr after burning the adsorption of dye increases sharply (up to 30 per cent) both in the burnt and in the adjacent parts of the cornea, after which the colour in the latter falls to 70 per cent of the normal (control) cornea and then increases again, the adjacent cornea absorbing for 4 days about the same amount of dye as the burnt (about 40 per cent more than the control). Romanov (1953a) also observed increased sorption of neutral red by the nerve cells of the cerebrum and spinal cord ganglia of rats under the influence of conditioned reflex excitation.

Zhirmunskii (1954, 1955) showed that in the first days after cutting the sciatic nerve of rats the denervated muscles are stained by neutral red and phenol red less strongly than the control muscles, but in the subsequent days more strongly. As a result of the denervation of one group of muscles there are changes in the colouring properties of the adjacent muscles which have not been denervated as in the cornea of the eye.

The work of Lobashev and Korenevich (1947) and Lobashev (1949) on frog muscles and also that of Romanov (1948b, 1949b, c, d, 1950, 1951, 1953b) on isolated rabbit spinal cord ganglia, on the nerve cells of mouse cerebrum (in experiments on the whole organism) and on frog muscles and yeast cells, showed that at certain times after the action of irritants there is lowered sorption of dyes by those cells in comparison with the control cells which were not subjected to any such excitation. After longer periods this subsequent staining effect disappears, and the cells become stained in the same way as the controls. Similar results were obtained in work by Smit-ten (1948) and Ushakov (1950). According to the observations of Romanov, at the stage when affected cells are less strongly stained, they are more stable to the action of certain damaging forces.

Suzdal'skaya (1959) showed that, when the excitability of isolated frog muscles is increased (by removal of the calcium from the Ringer's solution) by 30–80 per cent, the sorption of neutral and phenol red by them is lowered by 16–22 per cent.

Increased separation of granules of vital dyes in cells under the action of a great variety of excitants in weak doses (sub-threshold level) has been observed by many workers, such as: Fedorov (1934) in the case of acidosis, hypotony and mechanical excitation of the parasympathetic ganglia of frog hearts; Makarov (1936) exposing the same tissue to sub-narcotic concentrations of ether, chloroform and alcohol, and Kotlyarevskaya and Boldyrev (1939) on excitation of the vagus; Ries (1937) in the epithelial cells of axolotl skin under the action of ammonia, potassium cyanide, alcohol, hypotony and asphyxia and the cells of Leidig when subjected to the action of 0·01 per cent potassium cyanide solution. The same was seen by Krasil'nikova (1954) when she exposed the epithelium of mouse kidney tubules to weak doses of strychnine, by Romanov (1946, 1949a) in spinal cord ganglia of rabbits in the first stage of shock and on short-term excitation by induction currents through the nerve terminals and by Ushakov (1949) in the spinal cord ganglia of frogs. Rayevskaya (1948) also observed increased granule formation as damage spread along the muscle fibre, the zone of damage with diffuse colouring being preceded by the zone of increased granule formation. Murtazi (1950) established the presence of increased granule formation in the process of gastrulation in the peridermal cells of groundling embryos.

Nasonov and Aleksandrov (1940) consider phenomenon of the increased ability of the protoplasm to form granules of vital dyes in response to the

action of weak doses of excitants and reduced sorption of vital dyes in these conditions as an expression of the excited stage of narcosis. It may be supposed that, in response to the weak action of some altering agent or other, certain biochemical reactions are started in the cell which lead to the restoration of the initial state of the live matter, one expression of which may be reduced sorption of dyes by the protoplasm and simultaneous increased granule formation (Nasonov and Aleksandrov, 1940; Ushakov, 1949, 1951; Romanov, 1951; Nasonov, 1959a, etc.).

### *3. The Mechanism of the Distribution of Vital Dyes between Cells and their Surrounding Medium*

From the review of the literature which has been given in this chapter we come to the indubitable conclusion that the distribution of vital dyes, both basic and acid, and likewise the amount of these substances absorbed by the cell is directly dependent on the functional state of the cell or tissue. We saw that the most varied irritants, like the normal process of excitation, lead to a sharp change in the picture of the distribution of dyes in the cell and to a change in the ability of the protoplasm to absorb these substances from the surrounding medium.

The supporters of the membrane theory of cell permeability have until very recently treated the change in the colorability of the protoplasm upon the action of irritants on the cell as a consequence of the change in the permeability of the semi-permeable membrane—weak staining of the protoplasm and nucleus by vital dyes is explained by the fact that the semi-permeable membrane passes dyes only with difficulty, while the increased staining of the cells is seen as an increase in their ability to pass the relevant dyes (Höber, 1909, 1926, 1945; Gellhorn, 1929; Gutstein, 1932, and many others). However, all the results quoted in this chapter speak against a membrane mechanism regulating the passage of dyes into the cell.

From the point of view of the membrane theory it is impossible to understand why the action of some excitant or other on the cell causes the nucleus to be stained intensely before the cytoplasm, which remains for some time after as lightly stained as the cytoplasm of the control cells. Clearly, the passage of the dye into the nucleus cannot in this case be explained by an increase in the ability of the cell membrane to pass the dye because in that event it is the cytoplasm that should first be stained, and then the nucleus. Evidently what has happened is some change in the nucleus first, as a result of which the dye began to be concentrated there, passing through the whole thickness of the protoplasm in the process without staining it. Consequently, the absence of dye in noticeable quantities in the cytoplasm cannot be used as proof that its surface is impermeable to this substance. This fact indicates that dye can pass freely into the cell and accumulate in one or other of its structural elements depending on their sorptional activity. This is further

confirmed by the fact that the diffuse staining of the cytoplasm and nucleus coincides with a change in the colloidal properties of the live matter (gelatinisation, appearance of structures and so on).

Another fact militating against the membrane theory is that the cell in the paranecrotic state with strongly coloured cytoplasm and nucleus loses dye in the same solution of the dye if the agent causing that state is removed. This was admirably demonstrated by Aleksandrov (1932), Nasonov (1932a), Nasonov and Aleksandrov (1937) and other workers. If the increase in colorability was due to damage to the membrane, then it is impossible to see why, when the agent which "damaged the membrane" has been removed, the dye begins to move out of the cell. If there was a membrane at the bottom of the whole affair, then it could only prevent the issue of dye from the cell, because after the removal of the altering agent, as the supporters of the membrane theory think, its properties return to normal and it should as it were slam the door on the dye and prevent its issue from the cell.

From the sorptional point of view of regarding the phenomena of cell permeability (Nasonov and Aizenberg, 1937; Nasonov and Aleksandrov, 1937, 1940, 1943; Kamnev, 1938; Aleksandrov, 1939a; Nasonov, 1939, 1949a; 1959a; Troshin, 1953a, etc.), there is no difficulty in explaining the facts quoted in this chapter. From this point of view, dyes penetrate cells with difficulty and in small amounts not because there is some semi-permeable membrane preventing their entry, but because the sorptional properties of the whole mass of the protoplasm in relation to these substances are normally low. When subjected to the action of some altering agent (irritant) the protoplasm begins to absorb dyes in large quantities from the medium not because the semi-permeable properties of the membrane have been broken down making it more permeable, but because the action of the external agent has caused an increase in the sorptional properties of the whole mass of live matter, as a result of which the cell begins to absorb dyes in large amounts from the medium. As soon as the action of the altering agent is removed and the cell returns to normal, or when physiological activity is replaced by rest, the sorptional properties of the cell, thanks to the energy of metabolism, are lowered again and the dye which earlier passed into the cell now passes out of it. From this point of view it is also easy to understand the observed redistribution of dyes between the cytoplasm and nucleus, between the wall of plant cells and the protoplasm, between the latter and the contents of the vacuole, and so on.

This can also obviously be used to explain the fact that, when altering agents act on cells that were previously stained with granular dye, this dye is observed to come out of the granules with resultant diffuse colouring of the cytoplasm and nucleus (Shtutina, 1939; Shtempel' and Troshina, 1959).

As is well known, Nasonov and Aleksandrov (1940) consider that denaturising changes of the protoplasmic proteins are fundamental in the paranecrotic changes of live matter. The denaturation of native proteins, as is

shown in papers by Nasonov and Aleksandrov (1934, 1937, 1940), Aleksandrov and Nasonov (1939), Aleksandrov (1947, 1948 a, b, c), Braun (1948 a, b, 1949, 1951) and Golovina (1949), is in many respects reminiscent of paraneurotic changes and takes place owing to the same agencies as cause the paraneurosis of any cells. The agents which cause paraneurosis act on the cell in the same way as physiological irritants. In the opinion of Nasonov and Aleksandrov, denaturising changes in the cell proteins are fundamental in the process of interaction between the irritant and the live system. It is evidently with just these changes in the protoplasmic proteins that are connected the distribution and redistribution of vital dyes between cell and medium and, it may be thought, of many other substances as well (see Aleksandrov, 1959; Nasonov, 1959 a; Braun and Nemchinskaya, 1960).

In recent years Braun (1948 a, b, 1949, 1951) has published many new facts supporting the thesis that it is denaturational changes in the proteins of the protoplasm that are responsible for the changes in the colouring properties of live matter. He showed that the most various native proteins when isolated from the tissues of the organism increase their affinity for basic and acid dyes under the action of the same agents as cause paraneurosis.

Convincing proof of this thesis is given by the interesting experiments made by Braun (1949) with myosin threads. He obtained the threads from the myosin of rabbit muscles and stained them with various dyes while subjected to sounds of various frequencies (from 200–5000 c/s) but of the same intensity (about 115 dB). It turned out that the myosin threads, when subjected to sound, began to bind the dye more intensely than the same threads when not subjected to the action of sound, which indicates that denaturation of the myosin was beginning owing to the action of sound. It is interesting that the ability of myosin to react to sound is well marked in freshly prepared protein and disappears when it is kept (cold) for 1–2 days. The curve of the staining of the myosin threads in dependence on sound frequency reproduces almost exactly the curve for the staining of the muscles. Here the maximum staining lies also in the region of 2500–3000 c/s. A visual presentation of the effect of sound on the binding of vital dyes by muscles, spinal cord ganglia, epithelium of the kidney tubules and myosin threads is given in Fig. 77, taken from a paper by Nasonov and Rozental' (1950).

Nasonov and his colleagues connect paraneurotic changes in cells with the initial stages of the denaturation of the cell proteins. These denaturational changes are reversible in the conditions of the live cell. The increased binding by the protein of acid and basic vital dyes indicates that on denaturation free basic and acid groups are liberated in the protein molecule.

The enhanced staining of the protoplasm by basic dyes in reversible damage depends, further, on the appearance in the protoplasm of free nucleic acids, ATP etc., which normally form part of the protein complexes

of the protoplasm—nucleoproteins (Larionov and Brumberg, 1946; Larionov, 1948; Trifonova, 1948, 1952a, b; Kedrovskii, 1945, 1951; Aleksandrov, Manoilov and Orlov, 1952; Trifonova and Tikhomirov, 1952; Braun and Nemchinskaya, 1958, Nemchinskaya, 1959, 1960, and others).

A detailed explanation of the mechanism of the distribution of vital dyes between cells and their ambient medium is of great interest because the intensity and nature of the staining of the protoplasm, as we have seen, is a sensitive and easily observable indication of the functional state of the cell.

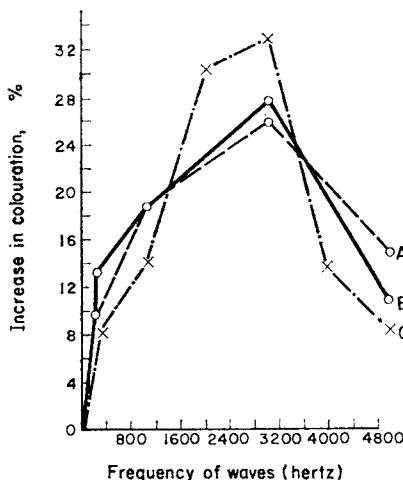


FIG. 77. The effect of audible sounds of varying frequencies on the coloration by neutral red of frog muscles (A), myosin fibres (B) and rabbit neuro-spinal ganglia (C) (from Nasonov and Rozental', 1950).

The study of the mechanism of distribution of vital dyes is also important because in this case it is easy to observe the localisation of the dye in the cell. In evaluating the significance for physiology of experiments with these chemical compounds it must be borne in mind that the mechanism of their distribution is probably the same as that set in motion by the cell on the absorption of nutritive and other substances (Nasonov and Aleksandrov, 1934; Guilliermond, 1941; Meisel', 1950; Nasonov, 1959a, and others).

In contrast to the substances whose distribution we were discussing in the preceding chapters, vital dyes are organic electrolytes of large molecular size.

The writer (1952) has studied the distribution of the following dyes: (1) neutral red, a basic vital dye, which *in vivo* stains muscle fibres diffusely with the formation in the sarcoplasm of a small number of tiny granules; (2) rhodamine, also a basic dye, which stains muscle fibres less diffusely (Fel'dman, 1948a; Braun and Fel'dman, 1949); and (3) phenol red, an acid dye, which stains muscle fibres diffusely.

Prepared frog sartorius muscles were kept before the experiment for 1·5–2 hr in Ringer's solution, then immersed in a solution of the dye made up in Ringer's solution. The solution of neutral red was prepared in Ringer's solution from which sodium had been omitted. After a fixed time interval the muscles were taken out of the dye solution, dried with paper, their central portions were cut out, these being subsequently weighed on a torsion balance, after which the dye was extracted from them with 70 per cent alcohol made acid with sulphuric acid. Next a Pulfrich photometer was used to

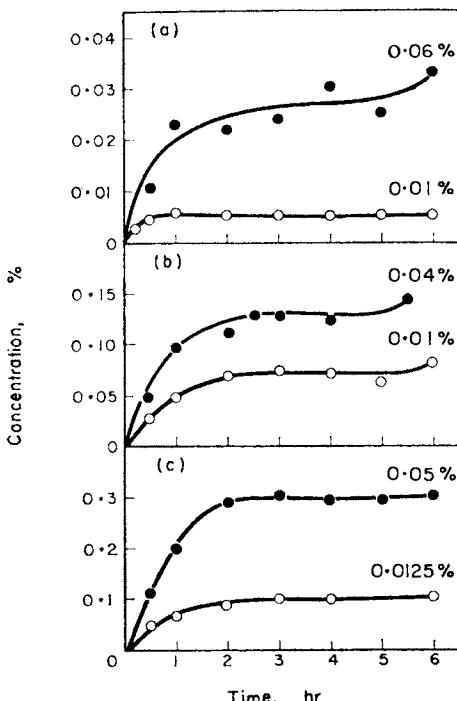


FIG. 78. The absorption of vital dyes from the medium by frog sartorius muscles with time. The concentrations of the dyes in the surrounding solution are indicated above the curves. The dye concentrations in the muscles along the ordinates are shown in per cent (in g per 100 ml of water of whole muscle).

a—phenol red, b—neutral red, c—rhodamine.

determine the amount of dye absorbed by the muscle. The concentration of dye in the medium and muscles was calculated in grams per 100 ml of water. For comparison parallel experiments were made with muscles killed by 5 min immersion in Ringer's solution that was heated to 80°. A third series of experiments was made at 34° C. This is the temperature above which frog muscles become more stainable by vital dyes (Butkevich-Troshina, 1948).

First of all the time was determined at which diffusion equilibrium was achieved between the dye absorbed by the muscle and the medium. The results of these experiments are shown in Figs. 78 and 79: they indicate that diffusion equilibrium between live muscles and the medium in the experiments with phenol red is reached after 1 hr, and for neutral red in 0·04 and 0·01 per cent concentrations and rhodamine in 0·05 and 0·0125 per cent concentrations significantly later—after 2–3 hr. Equilibrium between dead muscles and the solutions of neutral red and rhodamine in the same concentrations is reached after 20–30 hr and with the phenol red solutions after 10–15 hr.

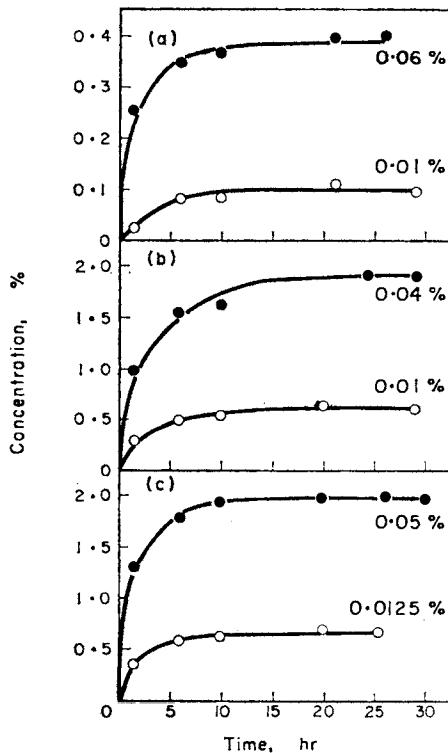


FIG. 79. The absorption of vital dyes from the medium by dead frog sartorius muscles with time. (For key, see legend to Fig. 78)

The time needed for the establishment of diffusion equilibrium in the experiments at 34°C could not be established, because in this case the sorption of dye by the muscles increases all the time. This increase in sorption continues right up to the death of the tissue. In long-term experiments at this temperature thermal effects and the dye probably acquire the status of damaging agents.

In the equilibrium state there is approximately 50–60 per cent less phenol red in live muscles than in the medium, whereas the equilibrium concentration of neutral red and rhodamine is many times greater.

Under the microscope this difference in the staining is very striking: the sarcoplasm of the muscle fibres is uniformly stained very weakly by phenol red and extraordinarily intensely by neutral red and rhodamine. It might be supposed that such a strong contrast in staining was connected with damage

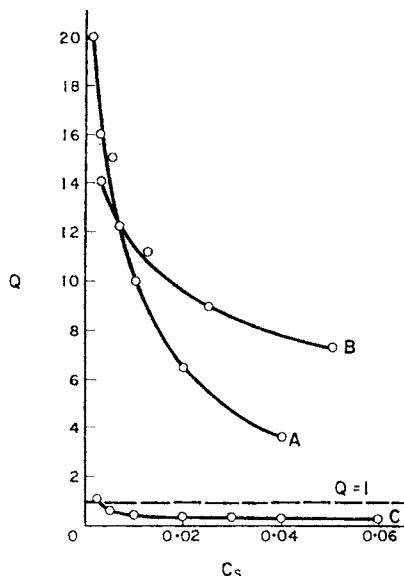


FIG. 80. The dependence of the coefficient  $Q$  on the concentration of dyes in the medium ( $C_s$ , in per cent) on the distribution of vital dyes between frog muscles and medium at 19–20°.

A—neutral red, B—rhodamine, C—phenol red.

of the sarcoplasm in the solutions of the basic dyes, but this is not the case. Both in the solutions of phenol red and in the rhodamine the excitability of the muscles does not differ for 24 hr from that of the muscles which were kept for this time in pure Ringer's solution. The excitability of the muscles in the solution of neutral red falls noticeably, but in this dye too it changes insignificantly in the first 6 hr. These observations are in good agreement with the results of Fel'dman (1948 a), who made a special study of the toxicity for isolated frog muscles of a number of dyes.

In the following three series of experiments a study was made of the dependence of the concentration of vital dyes in the muscles on the concentration in the surrounding medium. The length of the experiments with the live muscles at room temperature and at 34°C was 3 hr, with the dead mus-

cles 29–30 hr. As can be seen from Figs. 78 and 79, this period was adequate for the establishment of complete diffusion equilibrium (see also V. B. Ushakov, 1961 a, b).

Upon analysis of the results obtained the immediately striking features are: first, the difference in the capacity of live and dead muscles to absorb acid and basic dyes; second, the discovery of the same law governing the distribution of the dyes as was seen in the case of non-electrolytes, amino acids and other substances; and third, the sharp change in the sorptional

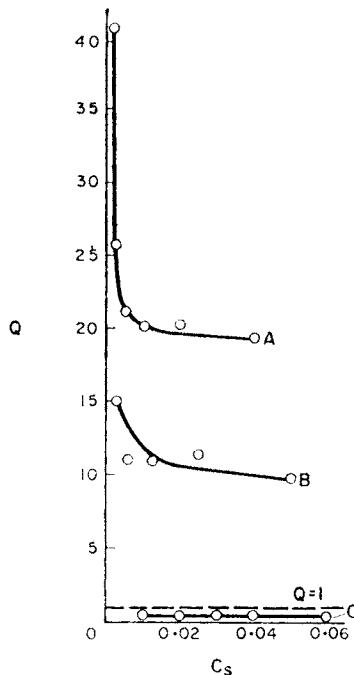


FIG. 81. The dependence of the coefficient  $Q$  on the concentration of the dyes in the medium ( $C_s$ , in per cent) on the distribution of vital dyes between frog muscles and medium at elevated temperature (34°).

A—neutral red, B—rhodamine, C—phenol red

properties of muscles in relation to acid and basic dyes upon elevation of the temperature and upon their destruction.

Thus, if the equilibrium concentration of neutral red and rhodamine in the live muscles in all the concentrations tried is many times greater than in the medium, then for phenol red in the same concentrations it is half to a third of that level: it is only at very low concentrations that  $C_c$  becomes greater than  $C_s$ .

This difference in the sorption by the muscles of basic and acid dyes is maintained in elevated temperature conditions too and also in the staining of the dead muscles, as can well be seen from Figs. 80, 81 and 82.

TABLE 46. THE RATIO ( $Q$ ) OF THE CONCENTRATIONS OF DYES IN MUSCLE ( $C_c$ ) TO THEIR CONCENTRATION IN THE MEDIUM ( $C_s = 0.02$ )

Experimental conditions	Phenol red	Neutral red	Rhodamine
Live muscle at 18–20°C	0.35	6.56	9.50
Live muscle at 34°C	0.55	20.30	10.05
Dead muscle at 18–20°C	11.40	59.85	54.90

For comparison, we show in Table 46 the values of  $Q$  obtained for all three dyes in conditions of diffusion equilibrium at a concentration in the medium  $C_s = 0.02$  per cent in the experiments with the live muscles at room temperature and at 34°C, and also in the experiments with the dead muscles.

This table shows that the sorptional capacity of live and dead muscles differs for different dyes. There is a particularly large difference in the sorption of acid and basic dyes. The amount of phenol red in live muscles is 50–67 per cent less than in the medium, while that of neutral red is many

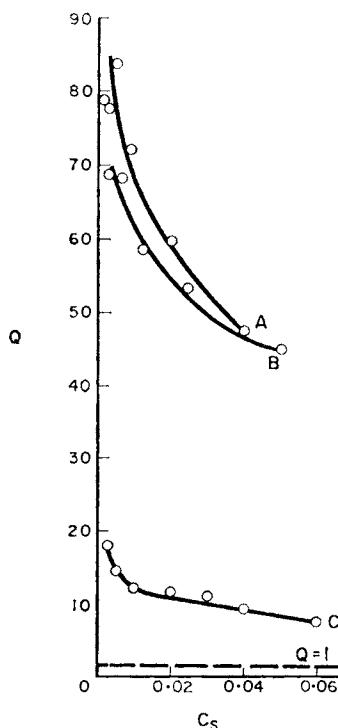


FIG. 82. The dependence of the coefficient  $Q$  on the concentration of dyes in the medium ( $C_s$ , in per cent) on the distribution of vital dyes between dead frog muscles and medium.

A — neutral red, B — rhodamine, C — phenol red

times greater. Dead muscles absorb both phenol red as well as neutral red and rhodamine in such quantities that in the equilibrium state their concentration in the muscles exceeds that in the medium by a factor of 10·4 (phenol red) and by 55–60 (basic dyes).

Reversible damage to the muscles (staining at 34°), and irreversible as well, in all cases leads to a strong increase in the sorptional properties of the muscle tissue, though this increase is not the same for the different dyes—it is greatest of all for phenol red and least for rhodamine. This follows from the data in Table 47, where it is shown how many times the sorption of dyes by damaged and dead muscles exceeds the sorption by normal muscles.

TABLE 47. THE INCREASE IN THE SORPTION OF DYES BY MUSCLES WHEN DAMAGED. THE EQUILIBRATED CONCENTRATION OF THE DYE IN THE MEDIUM  $C_s = 0.02$  per cent; THE QUANTITY OF THE DYES ABSORBED BY NORMAL MUSCLES IS TAKEN AS UNITY

Experimental conditions	Increase in sorption of		
	Phenol red	Neutral red	Rhodamine
Live muscle at 18–20°	1.0	1.0	1.0
Live muscle at 34° (damaged)	1.6	3.1	1.1
Dead muscle at 18–20°	32.6	9.1	5.8

Thus, the greatest increase in staining of damaged muscles (at 34°) occurs in the case of neutral red, the smallest for rhodamine.

Irreversible damage of the muscles leads to very strongly increased sorption of all dyes, but the increase is greatest for the acid dye.

The dependence of  $Q$  on the concentration of the dyes in the surrounding equilibrated liquid, indicated by the curves in Figs. 80, 81 and 82, shows clearly that the smaller the concentration of dye in the surrounding fluid ( $C_s$ ), the greater the value of  $Q$ , i.e., the percentage absorption of dye from the medium increases in inverse proportion to the concentration in the medium. This holds for both live and dead muscles.

This, as we have seen, is the same kind of dependence of  $Q$  on  $C_s$  as is observed in the distribution of sugars, amino acids, creatinine, urea and other substances, and it is evidently due to the same causes.

The contributions made by adsorption and solution to the distribution of vital dyes between muscles and medium are not the same. They depend both on the nature of the dye itself and on the concentration. The results obtained allow us to make an approximate calculation of the proportion of the total amount of dye passing into the muscle which can be attributed to solution and adsorption respectively. For phenol red this split can be made sufficiently accurately, as was done in the case of sugars, amino acids and creatinine. In Figs. 83, 84 and 85 the curves A, B, C show the dependence of the concen-

tration of vital dyes in the muscle fibres on their concentration in the surrounding fluid. In these figures the line OE corresponds to equal concentrations of dye in the medium and in the muscle fibres ( $C_c = C_s$ ). The actual line of the distribution curves differs sharply from this straight line. The distribution curves for neutral red and rhodamine in all three cases lie

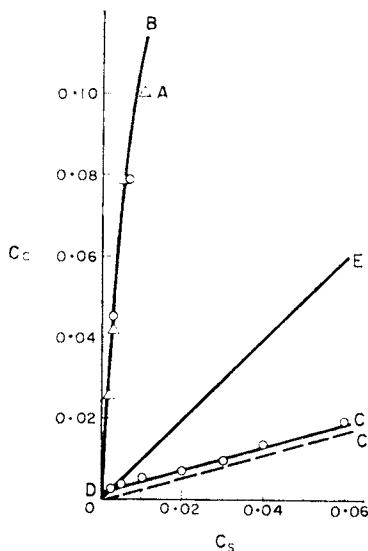


FIG. 83. The dependence of the concentration of vital dyes in the muscle fibres ( $C_c$ , in g/100 ml of intracellular water) on their concentration in the medium ( $C_s$ , in per cent) at 19–20°.

A—neutral red, B—rhodamine, C—phenol red. The line OE corresponds to equality of the concentrations in the medium and the muscle fibres ( $C_c = C_s$ ).

considerably higher than the line OE, which indicates strong binding of these substances by the muscle colloids. The distribution curve of phenol red for dead muscles has the same shape, but the curve for live muscles recalls rather the similar curves for sugars, amino acids and creatinine.

All the distribution curves for dead and live muscles obey the formula given above:  $C_c = C_s K + A$ , but only the curve for phenol red in live muscles permits the graphical determination of the constant  $K$  and the quantity  $A$  in the formula (see Fig. 83).

For phenol red in the experiments with live muscles the quantity  $A$  remains constant over the whole range of concentrations tested: it is equal to the intercept on the ordinate OD, which corresponds to the adsorption limit, and is equal to about 0.001 g of dye per 28.6 g dry residue of muscle (or 100 ml intracellular water). The straight line OC<sub>1</sub>, parallel to DC, reflects the dependence of the dissolved dye in the muscle fibre ( $C$ ) on  $C_s$  and obeys the equation  $C = C_s K = C_c - A$ , where  $K = 0.25$ .

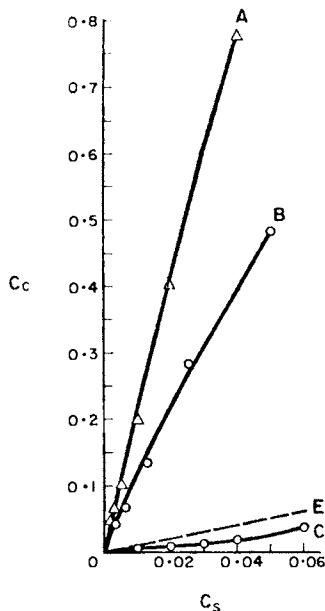


FIG. 84. The dependence of the concentration of vital dyes in muscle fibres on their concentration in the medium at elevated temperature (34°).

For key, see Fig. 83.

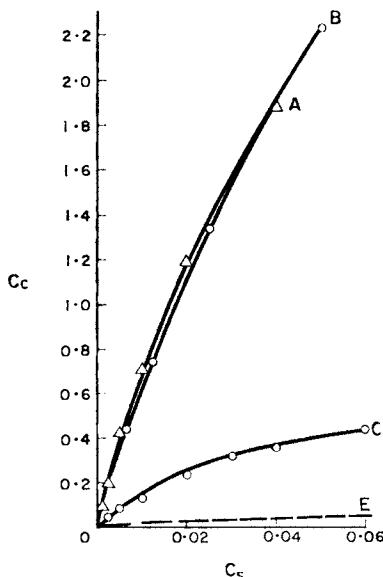


FIG. 85. The dependence of the concentration of vital dyes in dead frog muscles on their concentration in the medium.

For key, see Fig. 83.

The distribution coefficient  $K$  indicates that, independently of the concentration of phenol red in the medium, 75 per cent less of this substance is dissolved in the muscle fibre than in the surrounding solution. It is not possible to determine the value of the distribution coefficient  $K$  for neutral red and rhodamine (and also in the experiments with phenol red in dead muscles)

TABLE 48. THE CONCENTRATION OF DISSOLVED AND ADSORBED VITAL DYES IN THE MUSCLE FIBRES OF LIVE FROG MUSCLES. TEMPERATURE 18–20°C.

No.	Concentration of vital dyes (g per 100 ml water)		Of the total amount of dyes in the muscle fibres ( $C_c$ ) there is (in g per 100 ml water)		Percent dissolved of total amount of dye in the muscle fibres	$Q = \frac{C_c}{C_s}$
	in medium ( $C_s$ )	in muscle fibres ( $C_c$ )	in dissolved state ( $C = C_s K$ )	in adsorbed state ( $A = C_c - C$ )		
Phenol red						
1	0.06	0.0196	0.0150	0.0046	75	0.33
2	0.04	0.0129	0.0100	0.0029	77	0.32
3	0.03	0.0092	0.0075	0.0017	81	0.31
4	0.02	0.0070	0.0050	0.0020	71	0.35
5	0.01	0.0051	0.0025	0.0026	50	0.51
6	0.005	0.0033	0.0012	0.0021	37	0.66
7	0.0025	0.0027	0.0006	0.0021	22	1.08
Neutral red						
1	0.04	0.142	0.0200	0.1220	14.0	3.73
2	0.02	0.131	0.0100	0.1210	7.6	6.56
3	0.01	0.104	0.0050	0.0990	4.8	10.40
4	0.005	0.078	0.0025	0.0755	3.2	15.60
5	0.0025	0.042	0.0013	0.0407	3.1	16.08
6	0.0012	0.026	0.0007	0.0253	2.7	20.80
Rhodamine						
1	0.05	0.370	0.0250	0.3450	6.7	7.45
2	0.025	0.228	0.0120	0.2155	5.5	9.12
3	0.0125	0.141	0.0063	0.1347	4.5	11.28
4	0.0063	0.078	0.0031	0.0749	4.0	12.38
5	0.0031	0.045	0.0016	0.0434	3.5	14.51

from the results of these experiments because the adsorption of dyes in these cases is well below the limit. By analogy with the distribution of sugars, alanine, creatinine and phenol red in live muscles it can be assumed that the distribution coefficient  $K$  for rhodamine and neutral red is also less than unity and, in any case, cannot be greater than this. The distribution coefficient  $K$  for all three dyes between dead muscles and the medium is probably

TABLE 49. THE CONCENTRATION OF DISSOLVED AND ADSORBED VITAL DYES IN THE MUSCLE FIBRES OF DEAD FROG MUSCLES. TEMPERATURE 18-20°C

No.	Concentration of vital dyes (g per 100 ml water)		Of the total amount of dyes in the muscle fibres ( $C_c$ ) there is (in g per 100 ml water)		Percent dissolved of total amount of dye in the muscle fibres	$Q = \frac{C_c}{C_s}$
	in medium ( $C_s$ )	in muscle fibres ( $C_c$ )	in dissolved state ( $C = C_s K$ )	in adsorbed state ( $A = C_c - C$ )		
Phenol red						
1	0.06	0.459	0.060	0.399	13.1	7.65
2	0.04	0.372	0.040	0.332	10.7	9.30
3	0.03	0.332	0.030	0.302	9.0	11.07
4	0.02	0.228	0.020	0.208	8.87	11.40
5	0.01	0.121	0.010	0.211	8.0	12.10
6	0.005	0.073	0.005	0.068	6.8	14.60
7	0.0025	0.045	0.0025	0.0425	5.6	18.00
Neutral red						
1	0.04	1.894	0.040	1.854	2.1	47.35
2	0.02	1.197	0.020	1.177	1.7	59.85
3	0.01	0.723	0.010	0.713	1.4	72.30
4	0.005	0.425	0.005	0.420	1.2	85.00
5	0.0025	0.195	0.0025	0.1925	1.3	78.00
Rhodamine						
1	0.05	2.251	0.050	2.201	2.2	45.02
2	0.025	1.345	0.025	1.320	1.8	53.80
3	0.0125	0.733	0.0125	0.7205	1.7	58.64
4	0.0063	0.432	0.0063	0.4257	1.5	68.57
5	0.0031	0.209	0.0031	0.2059	1.4	67.78

equal to unity, because it is difficult to suppose that the solubility of substances in the water of dead muscles is substantially different from their solubility in the water of the surrounding medium.

In Tables 48 and 49 we show the concentrations of dyes in muscle fibres at various different concentrations in the surrounding medium and also indicate the two fractions (dissolved and adsorbed) of the total amount of dye in the muscles. For the determination of the dissolved fractions it was assumed that  $K$  for neutral red and rhodamine in the experiments with live muscles was 0.5; for phenol red, as we have seen,  $K = 0.25$ . The distribution coefficient  $K$  for all three dyes in the experiments with dead muscles was taken to be unity.

It follows from the tables that for rhodamine and neutral red only an insignificant amount (from 14 to 2.7 per cent for live muscles and from 2.2 to 1.3 per cent for dead muscles) is to be attributed to the dissolved dye

in the muscles. The great mass of these dyes in the muscle is there in the adsorbed state. The same applies to phenol red in dead muscles too. This is the explanation of the sharp rise in the distribution curves over the line OE which can be seen in Figs. 83 and 85. For phenol red in the experiments with live muscles the proportion of dye dissolved in the live muscle fibre varies within wide limits depending on the concentration of dye in the medium, namely: for  $C_s$  from 0·06 to 0·03 per cent 75–81 per cent of the total dye present in the muscle is dissolved in the muscle fibre, while, as  $C_s$  falls, this percentage drops, reaching 22 per cent when  $C_s = 0\cdot0025$  per cent. However, when  $C_s = 0\cdot0025$  per cent the equilibrium concentration in the muscle is greater than that in the surrounding liquid ( $Q = 1\cdot08$ ), in spite of the fact that its solubility in the sarcoplasm is a quarter of that in the water of the surrounding medium ( $K = 0\cdot25$ ). This excess of  $C_e$  over  $C_s$  in the present case is to be attributed to adsorption.

Thus, on the basis of the results we have quoted we may conclude that there is no qualitative difference, or difference in principle, between the distribution mechanism for vital dyes and for sugars, alanine, creatinine and other substances. There are two factors which determine the distribution of these substances, great as are their chemical and physical differences: their solubility in the protoplasm and the adsorption (or chemical binding) by the cell colloids. The proportions adsorbed and dissolved in the distribution of any of these substances between cells and medium depend only on their concentration in the external medium. Whether the concentration in the cell is greater or less than the concentration of the substance in the medium also depends only on the concentration of this compound in the surrounding fluid.

#### 4. Conclusions

1. Plant and animal cells are permeable for a wide variety of dyes, both basic and acidic, both soluble in lipids and insoluble.
2. The absorption of vital dyes by the cell depends not only on the properties of a semi-permeable membrane, but on the sorptional level of the whole mass of live matter and, principally, on the state of the proteins of the protoplasm.
3. The amount of vital dyes which passes into the cell and also their distribution between the cell and the medium depends on the functional state of the protoplasm. This dependence consists in the fact that a change in the functional state of the cell is accompanied by a change in the sorptional capacity of the cell proteins.
4. Upon excitation and reversible damage of live matter dyes pass into the cell in great quantities, while, when the latter passes into the resting state, just as when the damaging agent is removed, these dyes pass from the cell to the outside.
5. The change in the sorptional properties of the cell proteins also explains the redistribution of dyes in the cell: the transition from the granular type of

staining to diffuse staining of only the nucleus under certain conditions, the movement of dye from the nucleus to the protoplasm under other conditions, and so on.

6. There is a deep analogy between the distribution mechanism for vital dyes and that for non-electrolytes, amino acids and other substances. The absorption by cells of either kind is determined by the sorptional properties of the protoplasm: by their lowered solubility in live matter and by the capacity of the cell colloids for adsorptional and chemical binding of them.

## CHAPTER VIII

# The Permeability of Cells for Mineral Substances

### 1. Introductory Remarks

*The mineral composition of cells and of their environment.* It is a well-known characteristic feature of any live cell that its mineral composition differs sharply from that of the medium. As a rule, the predominant cation in a cell is potassium, the concentration of which in the protoplasm is usually many times greater than in the extra-cellular fluid. For sodium the reverse concentration ratio holds good. Similarly anions are present in different amounts in the cell and in the medium. With the death of the cell this difference between the mineral composition of the live matter and the medium disappears. Much work has been devoted to the analysis of the electrolytes of cells and organs and also of the fluid bathing them. The most complete summaries of the literature on the problem are to be found in the works of Fenn (1936), Irving and Manery (1936), Kaplanskii (1938), Krogh (1946), Hodgkin (1951, 1958), Ling (1952, 1955, 1960), Manery (1954), Conway (1957), Epshtein (1957), Shanes (1958), Harris (1960) and others.

Here we need quote only a few characteristic examples to show the marked difference in mineral composition that exists between a cell and its surrounding medium.

TABLE 50. THE MINERAL COMPOSITION OF THE SERUM AND BLOOD CORPUSCLES  
OF THE RABBIT, BULL AND DOG (in g/kg of erythrocytes or serum)

(from Abderhalden, 1898)

Ions	Rabbit		Bull		Dog	
	Serum	Erythrocytes	Serum	Erythrocytes	Serum	Erythrocytes
Na <sup>+</sup>	3.292	—	3.195	1.654	3.159	2.090
K <sup>+</sup>	0.215	4.340	0.212	0.599	0.188	0.240
Ca <sup>++</sup>	0.082	—	0.085	—	0.081	—
Mg <sup>++</sup>	0.028	0.046	0.027	0.010	0.024	0.042
Cl <sup>-</sup>	3.883	1.236	3.690	1.813	4.023	1.352
PO <sub>4</sub>	0.085	2.305	0.113	0.466	0.106	0.726
H <sub>2</sub> O	925.600	633.530	943.640	591.860	923.980	644.260

In Table 50 we show part of the data given in a paper by Abderhalden (1898), which gives an impression of the mineral composition of erythrocytes and plasma of rabbits, bulls and dogs.

According to Abderhalden's analyses, rabbit erythrocytes contain absolutely no sodium. Numerous results reported by other writers show, however, that this cation is present in all cells, including rabbit erythrocytes, but in considerably smaller quantities than in the medium. Thus, according to Kerr (1937), rabbit erythrocytes contain 16 mM/kg of sodium and 99 mM/kg of potassium.

It can be seen from Table 50 that in rabbit, bulls and dog blood the concentration of potassium, phosphate and magnesium in the erythrocytes exceeds that in the serum, whereas there is in all cases more sodium and chlorine in the plasma than in the cells. In the blood of bulls and especially dogs the difference between the sodium and potassium concentrations in the serum and erythrocytes is less sharply marked than in other animals, the predominant ion here being not potassium, as it is for most cells, but sodium.

Table 51 characterises the distribution of mineral ions between the formed elements and the plasma in human blood. Also in this table are given the cation concentrations in relation to the water content of the cells and plasma together with the ratio of those concentrations ( $Q$ ).

TABLE 51. THE DISTRIBUTION OF POTASSIUM, SODIUM, CALCIUM AND MAGNESIUM IN HUMAN BLOOD  
(from Hald and Eisenman, 1937)

Cations	Ion content (in mM/kg)				$Q = \frac{C_e}{C_s}$
	Serum	Serum water ( $C_s$ )	Erythrocytes	Erythrocyte water ( $C_e$ )	
K <sup>+</sup>	4.6	5.1	82.5	113.3	22.21
Na <sup>+</sup>	135.1	151.1	16.8	26.3	0.17
Ca <sup>++</sup>	10.6	11.8	0.4	0.62	0.58
Mg <sup>++</sup>	3.2	3.6	9.2	14.4	4.5

It can be seen from the table that in human erythrocytes there is more potassium and magnesium than in the serum, while, on the other hand, there is less sodium and calcium. These differences are particularly large for potassium and sodium.

Just as in the blood of animals, so in the erythrocytes and plasma of human blood, mineral ions are present in differing amounts (Table 52).

Just as in blood, mineral ions are distributed in unequal concentrations between tissue cells and their extracellular fluid. For example we cite in Table 53 some data from the work of Fenn and his colleagues.

It follows from the table that the concentrations of potassium, magnesium and phosphate in the muscles and nerves are greater than in the plasma, while the position is reversed for sodium and chloride.

In plant cells the distribution of mineral ions has been studied in detail in the case of the giant cells of sea and fresh water algae. True, in this case what is analysed is not the protoplasm but the cell juice. In certain algae, on which many experiments have been done, there is only a thin protoplasmic

TABLE 52. THE DISTRIBUTION OF MINERAL ANIONS IN HUMAN BLOOD  
(in mequiv/l.) (from Kaplanskii, 1938)

Anion	Plasma	Erythrocytes
Cl <sup>-</sup>	104	62·8
HCO <sub>3</sub> <sup>-</sup>	28	27·6
SO <sub>4</sub> <sup>2-</sup>	1	—
HPO <sub>4</sub> <sup>2--</sup>	2	3·6

TABLE 53. THE CONCENTRATION OF MINERAL IONS IN THE PLASMA, SCIATIC NERVE AND MUSCLES OF THE FROG (in mm per 100 g of tissue or plasma, water in per cent of free weight of tissue). (From the data of Fenn, 1936; Fenn, Cobb, Hegnauer and Marsh, 1934/5)

Ions	Plasma	Nerve	Muscle
Na <sup>+</sup>	10·38	6·20	2·45
K <sup>+</sup>	0·25	4·80	8·30
Ca <sup>++</sup>	0·20	0·36	0·25
Mg <sup>++</sup>	0·30	0·80	0·83
Cl <sup>-</sup>	7·43	3·70	1·09
PO <sub>4</sub> <sup>3--</sup>	0·31	1·00	1·28
HCO <sub>3</sub> <sup>-</sup>	2·54	1·08	1·07
H <sub>2</sub> O	96·00	75·00	80·00

layer surrounding a large vacuole. In the opinion of Krogh (1946), such a protoplasmic film separating the contents of the vacuole from the external medium can be compared in respect of ion distribution mechanism not with individual animal cells, but with the tissue membranes of multicellular organisms, for example, with capillary walls, with the wall of the renal tubules, the barrier between the brain and the blood, and so on. The difference between the mineral ion concentration of the cell juice and the medium in which these algae live is very large (see Table 54).

In conclusion of this section it must be noted that the figures given which indicate a sharp difference in the concentrations of individual mineral ions in the medium and the cells are subject to very wide variations.

Many workers have shown that the ratio of the concentration of some ions, for example, sodium, potassium or chloride, in the cell to its concentration in the surrounding medium changes in the process of embryonic development and as the organism approaches old age (see Irving and Manery, 1936; Safarov, 1939; Widdas, 1954; Chalfin, 1956, etc.).

Koval'skii (1938, 1947), Safarov (1938), Koval'skii, Redina and Chulkova (1948), Koval'skii and Rayetskaya (1951) and Koval'skii and Chulkova (1951) observed a daily and seasonal variation in the mineral composition of the cells and tissues of vertebrates. As we shall see further, the distribution of mineral substances between cells and medium depends in a definite way both on the functional state of the organism as a whole and of its individual tissues and organs.

TABLE 54. THE MINERAL COMPOSITION OF THE CELL SAP OF CERTAIN ALGAE AND THE MEDIUM IN WHICH THEY LIVE (in mm) (from Osterhout, 1933)

Ions	Sea water	Valonia macrophysa	Halicystis	Saltish water	Chara ceratophylla	Pond water	Nitella
Cl <sup>-</sup>	580	597	603	73	225·0	0·9	90·8
SO <sub>4</sub> <sup>2-</sup>	36	traces	traces	2·8	3·9	0·3	8·3
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	0	—	—	traces	4·1	0·0002	3·6
NO <sub>3</sub> <sup>-</sup>	0	—	—	0·005	0·4	0·55	0
Na <sup>+</sup>	498	90	557	60	142·0	0·2	10·0
K <sup>+</sup>	12	500	6·4	1·4	88·0	0·05	54·3
Ca <sup>++</sup>	12	1·7	8	1·8	5·3	0·78	10·2
Mg <sup>++</sup>	57	traces	16·7	6·5	15·5	1·69	17·7

*Extracellular spaces.* In the study of the permeability of the cells which go to make up organs and tissues and also in the study of the distribution of substances between these cells and their environment, the determination of the size of the extracellular spaces, or, as they are often called, the "tissue spaces", is of great, and sometimes decisive, significance.

In certain cases, if the existence of these spaces is not taken into account, it becomes difficult to say whether some substance or other actually penetrates the cell or whether it is concentrated only in the tissue space. However, there are not at present any completely reliable methods for determining the size of these spaces.

The first measurements of the extracellular space of muscles were performed by Hermann (1888) from the difference between the muscle volume and the total volume of the muscle fibres.

At present the magnitude of the extracellular spaces is often determined from the distribution of certain substances between the tissue (or organ) and the surrounding liquid. If the substance does not penetrate the tissue cells

but is to be found only in the extracellular spaces, the ratio of its concentration per volume of total tissue to its concentration in the medium will give a figure indicating how much the tissue space is less than the volume of the tissue as a whole.

TABLE 55. THE MINERAL CONTENT IN HUMAN BLOOD SERUM AND ITS ULTRAFILTRATE (in mg per cent) (from Kaplanskii, 1938)

Medium	Na	K	Ca	Mg	Cl	P	S
Blood serum	250-350	16-22	9·4-13·2	1·8-3·9	320-400	1·8-4·7	2·0-4·0
Serum ultrafiltrate	250-350	16-22	4·0-6·0	0·8-1·8	350-450	1·8-4·7	2·0-4·0

It is considered that the extracellular spaces contain a liquid which is an ultrafiltrate of serum. In Table 55 we show data relating to the mineral composition of human blood serum and its ultrafiltrate. It can be seen that, with certain exceptions, these liquids differ little in their mineral composition.

Manery, Bale and Haege (Manery and Bale, 1941; Manery and Haege, 1941) proposed that the aqueous extracellular space of tissues should be determined from the distribution of "non-penetrating" ions according to the following formula (in ml of water per 100 g of fresh tissue):

$$(H_2O)_E \text{ of anion} = \frac{\text{Mequiv of anion/kg of tissue}}{\text{Mequiv of anion/1 of plasma}} \times 0.95 \times 0.93 \times 100,$$

$$(H_2O)_E \text{ of cation} = \frac{\text{Mequiv of cation/kg of tissue}}{\text{Mequiv of cation/1 of plasma}} \times \frac{0.93}{0.95} \times 100,$$

where  $(H_2O)_E$  is the aqueous extracellular space, 0.95 the Gibbs-Donnan coefficient and 0.93 a correction for the water content of the plasma. When radioactive substances are used, the ion concentration in the formulae is replaced by the number of impulses per unit weight of tissue and plasma in unit time.

According to Overton (1902a, b), all the chloride and sodium in tissues is present only extracellularly. In view of this, the extracellular space should occupy about 20 per cent of the muscle volume. Urano (1908) and Fahr (1909) immersed frog muscles for several hours in an isotonic sugar solution and found that 6 per cent in all of the total potassium content in the muscle and about 90 per cent of the chloride passes from the muscle into the solution. Sodium behaves similarly. On this basis the writers came to the conclusion that chloride is present only in the extracellular space, which should, according to their calculations, make up 14.5 per cent of the muscle volume.

The opinion that there is no chloride in muscle fibres is supported by many writers (Peters, 1935; Hastings and Eichelberger, 1937; Kometiani, Klein and Dolidze, 1946; Epshteyn, 1947, and others): they find confirmation

of this view in the small concentrations of this ion in muscle as compared with the concentration in the surrounding medium and in the quick transition of the chloride ion from the tissue into a solution containing no chloride.

Boyle, Conway, Kane and O'Reilly (1941) determined the extracellular space of frog sartorius muscles from the distribution of a number of substances. In their experiments they worked on the basis that, if chloride, sodium, magnesium and other ions, and also inulin, do not penetrate muscle fibres, then it would be possible, by placing the muscles for certain periods in solutions of these substances, to obtain in all cases a distribution coefficient of the same magnitude which would indicate the tissue space. However, it proved that the "magnesium", "chloride" and "inulin" extracellular spaces differ markedly from one another. The "magnesium" and "inulin" spaces make up 9·6 and 9·2 per cent, and the "chloride" space 12–13 per cent. Hence it follows that the "true" magnitude of the extracellular space is equal to about 10 per cent, and that part of the chloride and other ions penetrate not only into the tissue space, but also within the muscle fibres.

Taking all the chloride to be present extracellularly, Epshtein (1947) determined the extracellular space by this anion as 37–38 per cent of the tissue volume for the brain tissue of white rats and guinea pigs. According to Manery and Hastings (1939) and Manery and Bale (1941), the extracellular spaces of different tissues and organs of rats and rabbits vary within very wide limits, namely, from 11 to 35 per cent of the total tissue weight.

The size of the extracellular spaces, as determined from the chloride distribution, must be considered to be very strongly exaggerated, because it has been shown by many authors that chloride ions quickly penetrate cells and are normally constantly present in them.

In Table 56 we show the results of a number of authors who have determined the extracellular spaces of various animal tissues from the magnitude of the distribution coefficient for certain substances between tissue and blood plasma or between isolated tissues and artificial media replacing blood plasma. It can be seen that the extracellular spaces of different tissues differ in size very markedly one from another. Furthermore, for one and the same tissue the space for inulin, sucrose, mannitol, sorbitol, chloride ions and other substances varies, though the differences of one from another is not marked. Even the spaces for such substances as galactose, mannitol, sorbitol, which penetrate cells as we have seen, do not differ strongly from those for sucrose and chloride ions. The smallest extracellular space was found with inulin. If this substance really does fill only extracellular spaces, then all the other substances shown in Table 56 must penetrate inside cells.

Evidently, the "inulin space" is the nearest approximation to the true value of the extracellular space of tissue, but even this, as has been shown by Cotlove (1952, 1954), may be somewhat higher than the true value, because inulin is capable of slow penetration into cells (Tasker *et al.*, 1959).

TABLE 56. THE SIZE OF THE EXTRACELLULAR SPACES OF CERTAIN ANIMAL TISSUES  
(in per cent of the wet weight of the tissues or in ml of water per 100 g of fresh tissue)

Tissue	Substance used to determine the space	Size of space	Author
1	2	3	4
Frog sartorius muscle	chloride ions	14.7	Fenn, Cobb and Marsh (1935)
		17.7	Kometiani, Klein and Dolidze (1946)
	inulin	12.5	Desmedt (1953)
Toad sartorius muscle ( <i>Bufo marinus</i> )	sucrose	26.5	Tasker, Simon, Johnstone, Shankly and Shaw (1959)
	inulin	24.8	
	albumin	21.9	
Long extensor of frog IV finger	inulin	10.0	Keynes (1954)
Straight abdominal muscle of frog stomach	chloride ions	23.0	Kometiani, Klein and Dolidze (1946)
Frog gastrocnemius muscle	chloride ions	12.6	Kometiani, Klein and Dolidze (1946)
	sucrose (1%)	32.7	
	sucrose (4%)	28.4	
	galactose (2%)	42.1	
Smooth stomach muscle of frog ( <i>Rana pipiens</i> )	inulin	27.8	
	sucrose	34.0	
	fructose	42.3	
Sartorius muscle of rat	chloride ions	14.5	
of dog	chloride ions	15.5	
of guinea pig	chloride ions	15.5	
of cat	chloride ions	12.5	
of chicken	chloride ions	22.4	
Rat diaphragm muscle (isolated)	inulin	26	Creese (1954)
	inulin	14	Randle and Smith (1958a, b)
	thiosulphate	21-25	
	thiosulphate	20	Kipnis and Cori (1957)
	inulin	19	
	sucrose	30	Norman, Menozzi, Reid, Lester and Hechter (1959)
	mannitol	31	
Isolated tissues of eviscerated and nephrectomised rats:			
diaphragm	mannitol	20	
heart	mannitol	22	
gastrocnemius muscle	mannitol	16	
brain	mannitol	3	

TABLE 56—*continued*

Tissue	Substance used to determine the space	Size of space	Author
1	2	3	4
Rat heart muscle (perfusion)	inulin sorbitol sorbitol	30.5 37.8 35.6	Fisher and Lindsay (1956) Morgan, Randle and Regen (1959)
right auricle	inulin sucrose	24.8 32.9	Barclay, Hamley and Helga (1959)
left auricle	inulin sucrose	21.3 25.8	
right ventricle	inulin sucrose	15.1 22.4	
left ventricle	inulin sucrose	9.1 19.5	Barclay <i>et al.</i> (1959)
Cortical matter of rabbit kidneys (sections)	raffinose	33	Krane and Crane (1959)
Extracellular space of nephrectomised rats body skeletal muscle	raffinose raffinose	20 18	Helmreich and Cori (1957)
Extracellular space of rabbit body: normally after evisceration after evisceration and nephrectomy	glucose- <sup>14</sup> C glucose- <sup>14</sup> C glucose- <sup>14</sup> C	31 26 27	Wick, Drury and Mackay (1950)

Cotlove introduced inulin and sucrose into rat blood, maintaining the concentration of these substances in the plasma constant over a long period by repeated injections. After certain time intervals determinations were made of the inulin, sucrose and chloride concentrations in the skeletal muscles and the blood plasma. The basic results of Cotlove's paper (1954) are given in Table 57.

Similar results are reported by several authors (Nichols, Nichols, Weil and Wallace, 1952, 1953; Walser, Seldin and Grollman, 1954), who used the same procedure in experiments with dogs.

Cotlove's data show that the "chloride space" of rat skeletal muscle is about 12 per cent. Cotlove established that the inulin and sucrose space consists of two parts:  $\frac{2}{3}$  of this space is filled very quickly, but  $\frac{1}{3}$  very slowly. That part of the space which is slowly filled by inulin and sucrose does not belong to extracellular space. If we suppose that the maximum

TABLE 57. THE CHANGE WITH TIME IN THE INULIN, SUCROSE AND CHLORIDE ION "SPACE" IN RAT SKELETAL MUSCLE

(The spaces are given in ml of water per kg of fresh weight of tissue)  
(from Cotlove, 1954)

Time from beginning of introduction of sugars (hr)	Sugar space ( $V_{\text{sucrose}}$ )	Chloride space ( $V_{\text{chloride}}$ )	$\frac{V_{\text{suc.}}}{V_{\text{chlor.}}}$
Inulin			
1	95 ± 2.7	118 ± 3.1	0.81 ± 0.017
2	91 ± 1.7	113 ± 1.9	0.80 ± 0.011
6	92 ± 4.1	110 ± 1.9	0.85 ± 0.028
15	101 ± 4.2	110 ± 3.3	0.91 ± 0.016
15	93 ± 2.4	97 ± 2.5	0.97 ± 0.029
15	115 ± 7.1	125 ± 4.1	0.92 ± 0.033
average for 15 hr	106 ± 3.9	114 ± 3.1	0.93 ± 0.017
Sucrose			
2	98 ± 5.1	115 ± 4.8	0.85 ± 0.021
6	98 ± 3.2	115 ± 3.7	0.86 ± 0.019
15	110 ± 5.0	119 ± 3.9	0.93 ± 0.036
45	110 ± 5.0	114 ± 3.4	0.98 ± 0.057

inulin and sucrose space of muscle is equal to the chloride space, then the true extracellular space of inulin and sucrose will be 20 per cent less than the chloride, i.e., about 9.6 per cent of the wet weight of tissue.

Johnson (1955), using radioactive  $^{24}\text{Na}$ , sucrose- $^{14}\text{C}$  and sulphate- $^{35}\text{S}$ , found an unusually large value for the extracellular space of isolated frog muscles—about 40 per cent of the weight of muscle. There can be no doubt that this writer was using substances which penetrate muscle fibres. The present writer (Troshin, 1957), also using  $^{24}\text{Na}$ , found the extracellular space of this muscle to be 9.5 per cent. This value for frog sartorius muscle is lower than that given by Conway (1957), namely, 12.7 ml of water per 100 g of wet weight of tissue.

Tasker *et al.* (1959) showed that the size of the extracellular space of the sartorius muscles of the toad (*Bufo marinus*) varies strongly depending on the season of the year, changes strongly from one part to another of the animals, but is almost constant for paired muscles of one animal.

## 2. The Permeability of Animal Cells for Salts

*The permeability of erythrocytes for anions.* On the basis of osmotic experiments with the erythrocytes of various kinds of animals Hamburger (1891), Grijns (1896), Hedin (1897, 1898), Koeppe (1897), Kozawa (1914) and others came to the conclusion that the membrane of erythrocytes is

impermeable for all cations, though it is easily permeable for the majority of mineral anions.

This view of the properties of erythrocytes in respect of their permeability for ions finds no support in any quarter to-day. Chemical methods and the use of isotopes have established that mineral anions and cations penetrate into the erythrocytes of all kinds of animals and humans too. Only thanks to the use of radioactive isotopes as indicators has it become possible to establish that in the conditions of the organism and also in suitable conditions outside it there is a continuous exchange of every mineral ion in the cell for the same ion from the medium. Nevertheless, there is considerable asymmetry in the distribution of mineral ions between the cell and the surrounding medium.

Bernstein (1954) used modern methods to determine the concentration of ions of potassium, sodium, chloride and bicarbonates in the erythrocytes and plasma of a number of mammals. As can be seen from the figures in

TABLE 58. THE DISTRIBUTION OF POTASSIUM, SODIUM AND CHLORIDE IONS IN THE BLOOD OF A NUMBER OF MAMMALS

(The figures in square brackets show the ion concentration in mequiv/l. of cell water [ $n$ ], or of plasma [ ]<sub>e</sub>; those in round brackets the number of determinations)  
(from Bernstein, 1954)

Subject	Erythrocytes				Plasma				$\frac{[K]_i}{[K]_e}$	$\frac{[Na]_i}{[Na]_e}$	$\frac{[Cl]_i}{[Cl]_e}$	
	[K] <sub>i</sub>	[Na] <sub>i</sub>	[Cl] <sub>i</sub>	[K] <sub>e</sub>	[Na] <sub>e</sub>	[Cl] <sub>e</sub>						
Human (120)	136	19	78	5.0	155	112	27.4	0.6				1.44
Baboon (56)	145	24	78	4.7	157	115	30.8	0.15				1.48
Rabbit (15)	142	22	80	5.5	150	110	25.4	0.15				1.38
Rat (36)	135	28	82	5.9	152	118	23.0	0.18				1.44
Horse (8)	140	16	85	5.2	152	108	25.0	0.11				1.27
Sheep (18)	46	98	78	4.8	160	116	9.6	0.61				1.49
Bull (28)	35	104	85	5.1	150	109	6.8	0.69				1.28
Cat (5)	8	142	84	4.6	158	112	1.7	0.90				1.33
Dog (28)	10	135	87	4.8	153	112	2.1	0.88				1.44

Table 58, which is taken from this author's work, the potassium and sodium concentrations in erythrocytes vary strongly from one type to another, whereas the concentration of chloride ions remains about the same in all cases. The bicarbonate concentration also varies very little in humans and a number of animals. In his experiments with human erythrocytes Bernstein found that, when the pH was displaced towards the acid or basic side, the concentrations in the cells of chloride ions and bicarbonates changed quickly in accordance with Donnan's Law.

The causes of the unequal concentrations of each mineral ion in the cell and medium will be discussed in the following chapter. It may be noted here in the most general fashion that the constant asymmetric distribution of mineral ions occurs because the flux of an ion into the cell ( $m_{in}$ ) is equal to the flux in the reverse direction—from the cell into the medium ( $m_{out}$ ).

In isotonic salt solutions with polyvalent anions (sulphate, phosphate, tartrate, citrate, lactate etc.) erythrocytes shrivel up. According to Höber (1945) this is connected with the fact that the anions mentioned do not pass into the cell but cause only structural changes in the cell membrane—dehydration of the cell envelope. On the contrary, the anions  $\text{Cl}^-$ ,  $\text{OH}^-$  and  $\text{HCO}_3^-$  penetrate freely into erythrocytes and can displace one another in equivalent amounts (Van Slyke, Wu and McLean, 1923; Höber, 1945).

Maizels (1949) gave the following series of anions in order of decreasing speed of penetration into human erythrocytes (the speed of penetration of the chloride ion being taken as unity):

$\text{I}$  (1.24),  $\text{CNS}^-$  (1.09),  $\text{NO}_3^-$  (1.09),  $\text{Cl}^-$  (1.00),  $\text{SO}_4^-$  (0.21),  $\text{HPO}_4^{2-}$  (0.15).

Similar results are reported in articles by Woodhaus and Pickworth (1932) and Parpart (1940).

Univalent mineral anions pass into the erythrocytes of various animals and humans with incomparably greater speed than univalent cations, such as the potassium and sodium ions. Many writers have shown that change in the concentration of  $\text{CO}_2$  in the blood plasma leads to a very rapid redistribution of the  $\text{Cl}^-$  and  $\text{HCO}_3^-$  ions between the erythrocytes and plasma. The establishment of the new equilibrium:  $(\text{HCO}_3^-)_i/(\text{HCO}_3^-)_o = (\text{Cl}^-)_i/(\text{Cl}^-)_o$  takes only a few minutes altogether. According to the measurements of Dirken and Mook (1931), who developed a method for the rapid measurement of shifts in the distribution of these anions, the half-period  $t_{\frac{1}{2}}$  for the exchange of  $\text{HCO}_3^-$  for  $\text{Cl}^-$  is about 0.2 sec. According to Luckner (1939), who measured the change in the chloride ion concentration in a suspension liquid by means of silver chloride electrodes for sudden changes in the  $\text{CO}_2$  pressure, the emergence of chloride ions from the cell obeys a simple exponential law. He found that at 37° the half-period for the emergence of chloride ions from the cells  $t_{\frac{1}{2}}$  is 0.1–0.2 sec.  $Q_{10}$  for this process was 1.2–1.4.

The method of radioactive indicators has been used by many authors to study the speed of penetration of chloride and other halide ions (Love and Burch, 1953; Tosteson, 1956, 1959; Tosteson and Robertson, 1956, and others). Thus, Tosteson and Robertson (1956) found that isotopic equilibrium in a suspension of duck erythrocytes  $(\text{Cl}^-)_i/(\text{Cl}^-)_o = (\text{Cl}^{-38})_i/(\text{Cl}^{-38})_o = 0.74$ , is reached 3 min after mixing the erythrocytes with the radioactive liquid. Tosteson (1956, 1959) studied the permeability of human and bull erythrocytes for several halides by means of radioactive isotopes. In this work he used the method of Dirken and Mook (1931). The basic data from Tosteson's work (Tosteson, 1959) are given in Table 59. It can be seen that only the chloride ions in human erythrocytes are displaced according to a

simple exponential law, whereas in all the other cases the halide exchange process has two exponential components. In order of decreasing flux out of the erythrocytes the anions form the following series: Cl, Br, F, I. For bromide and iodide the ratio of the isotope concentration in the cell to that in the medium in conditions of isotopic equilibrium is greater than the corresponding quantity for chloride. This probably means that bromide and iodide

TABLE 59. THE DISTRIBUTION AND RATE OF EXCHANGE OF LABELLED HALIDES IN ERYTHROCYTES (from Tosteson, 1959)

Erythrocytes	Isotope	Equilibrium balance ratio of isotope concentration in cell to its concentration in the medium			Time constant of rate of ion release of slow fraction ( $\text{sec}^{-1}$ )
		fast fraction	slow fraction	total	
Bull	$^{38}\text{Cl}^-$	0.06	0.52	0.59	3.1
Human	$^{38}\text{Cl}^-$	0.00	0.54	0.54	3.1
Bull	$^{82}\text{Br}^-$	0.16	0.47	0.63	0.6
Human	$^{82}\text{Br}^-$	0.08	0.55	0.63	0.6
Bull	$^{18}\text{F}^-$	0.05	0.45	0.50	0.3
Bull	$^{131}\text{I}^-$	0.16	0.90	1.06	0.06
Human	$^{131}\text{I}^-$	0.12	1.09	1.21	0.07

ions can somehow be bound by erythrocytes. According to the author's calculations, the flux of anions into the erythrocytes and out of them is (in picomoles/cm<sup>2</sup> of cell surface/sec): chloride—13,000–13,900; bromide—3000, fluoride—2300; iodide—considerably lower than these values. These are exceptionally high speeds for ion exchange. The author notes that the chloride flux through the erythrocyte membrane is almost ten times greater than the sodium flux in the active axon of the cuttlefish, 100 times greater than the potassium flux out of resting cuttlefish fibre and 1000 times greater than the potassium flux out of resting frog muscle. It was found that bromide and iodide ions depress the flux of chloride ions out of erythrocytes.

Much evidence on the permeability of erythrocytes for the phosphate anion has been obtained by the method of labelled atoms. A complete summary of the literature on this problem is given in Hevesy (1951), Glynn (1957a) and Harris (1960). Hahn and Hevesy (1942) made a detailed study of the permeability of the erythrocytes of various animals for the phosphate anion. They introduced phosphate with an admixture of radioactive phosphorus into the blood of the animals. It was found that at body temperature in experiments outside the organism, for every 100 atoms of labelled P phosphate added to rabbit blood, after 1 hr 26 atoms had passed into 1 g of erythrocytes and 74 atoms remained still in 1 g of plasma. The exchange of phosphorus between the erythrocytes and plasma of human blood

was achieved with the same speed. The exchange of cell phosphorus for plasma phosphorus in chicken and frog blood is considerably slower.

Further, the authors established that the penetration of the radioactive chloride ion is accomplished at a speed 100 times greater than that of phosphate. In about 1–2 min the whole of the erythrocyte chloride is exchanged for plasma chloride. Even at 0° the greater part of this exchange procedure takes place in 1–3 min.

In an article by Hevesy (1951) a calculation is given of the rate of exchange of inorganic phosphorus in the erythrocytes for the plasma phosphorus in the case of human blood kept *in vitro* at body temperature. On the addition to the blood of a very small amount of radioactive sodium phosphate  $\frac{1}{3}$  of the labelled phosphorus atoms pass into the erythrocytes in 1 hr. On this basis it was calculated that of every 4 mg per cent of inorganic phosphorus in the plasma 0·7 mg passes from the plasma into the erythrocytes and as much again in the reverse direction. Experiments in identical conditions were made by Mueller and Hastings (1951) with human blood to which they added a small amount of doubly labelled potassium phosphate with an admixture of radioactive phosphorus. These authors found that on the average 8·49 micromoles of phosphorus pass from the plasma into the erythrocytes per litre of blood per minute.

Vladimirov, Pelishchenko and Urinson (1947, 1948), experimenting with human erythrocytes *in vitro*, established that at body temperature a considerable amount of labelled phosphate anion passes into the erythrocytes in exchange for unlabelled phosphate. If the temperature is lowered, the process is strongly inhibited.

Gourley and Matschiner (1953) in experiments with human erythrocytes *in vitro* found that in the exchange of erythrocyte phosphorus for the radioactive inorganic phosphorus of the plasma there is found to be a small quickly exchanging fraction ( $t_{\frac{1}{2}} = 8$  min) and a large slowly exchanging fraction ( $t_{\frac{1}{2}} = 280$  min). These two fractions are also found at a temperature of 3° (Pranker and Altman, 1954). The suggestion is made that the quick fraction is related to the phosphorus adsorbed on the surface of the cells.

A number of authors (Gourley, 1952; Pranker and Altman, 1954; Gerlach, 1955, 1956, etc.) made the suggestion that labelled inorganic phosphate passes into erythrocytes in the form of organic compounds in which it is already incorporated in the cell membrane.

The permeability of the erythrocytes of humans and also of several animals for sulphate has been established by chemical methods and by means of radioactive sulphate. Sulphate passes into these cells considerably more slowly than the univalent anions of a number of halogens (Parpart, 1940; Schweitzer and Passow, 1953; Passow, 1956).

*The permeability of erythrocytes for cations.* As has already been mentioned, osmotic experiments indicate the complete impermeability of erythrocytes

for cations. However, this view is refuted by a vast array of facts obtained in experiments both on whole organisms and outside the organism.

Thus, Davson (1934) showed that ox erythrocytes suspended in solutions of potassium chloride absorb potassium and lose sodium, whereas in solutions of sodium chloride the reverse process is observed. The presence in the solution of calcium chloride has no effect on the penetration of these cations into erythrocytes or out of them. Certain data from this paper are given in Table 60. They show clearly that ox erythrocytes in these conditions are permeable for both sodium and potassium.

TABLE 60. THE PERMEABILITY OF OX ERYTHROCYTES FOR POTASSIUM AND SODIUM  
(from Davson, 1934)

Washing fluid	Length of experiment (hr)	Concentration of sodium and potassium in erythrocytes (mg per cent)	
		potassium	sodium
Serum (normal)	—	83.2	159
1.48% NaCl	8	79.7	197
1.48% NaCl	30	79.6	218
1.48% NaCl	60	76.0	213
Serum (normal)	—	93.7	176
1.5% KCl	4.5	261	131
1.5% KCl	7.5	265	129
1.5% KCl	15.5	271	126
1.5% KCl	30.0	288	126

The author draws attention to the fact that in a 1.5 per cent solution of sodium chloride the sodium ion concentration in the erythrocytes increases rapidly. Equilibrium is attained after 5–10 min, but the concentration of this cation in the cells still does not reach the concentration in the surrounding medium. The potassium concentration in the erythrocytes also increases upon an increase in its concentration in the medium. In glucose solution there occurs a large loss of sodium from the erythrocytes and a small loss of potassium. The loss of sodium in this case is the same as in solutions of potassium chloride or in a mixture of potassium chloride and glucose.

Bakhromeyev and Pavlova (1935) upon stimulation of cat sciatic nerve observed a reduction of the potassium and calcium in the contracting muscles and an increase of these cations in the blood. At the same time it was found that an increase of potassium in the blood is accompanied by an increase of the potassium content of the erythrocytes. This means that red blood corpuscles are permeable for potassium.

Koval'skii and his colleagues (Koval'skii, Redina and Chulkova, 1948; Koval'skii and Rayetskaya, 1951; Koval'skii and Chulkova, 1951) found that the potassium, sodium, calcium and magnesium concentrations in erythrocytes vary in the course of 24 hr and that the erythrocytes of venous and arterial blood have different mineral ion concentrations. On the basis of these results the authors came to the conclusion that erythrocytes are permeable for the cations.

Numerous experiments with radio-isotopes have demonstrated the indubitable permeability of erythrocytes for cations. Thus, Hahn, Hevesy and Rebbe (1939 a, b) and Hevesy and Hahn (1941), introducing radioactive potassium and sodium chloride into the blood of rabbits and frogs, established the permeability of erythrocytes for these cations. Cohn and Cohn (1939) and Manery and Bale (1941) by introducing radioactive sodium into the blood of dogs also showed that the erythrocytes of this animal are permeable for sodium. It is important to note that according to their data the speed of penetration of sodium into the erythrocytes both in experiments in the whole organism and outside it was about the same.

Hahn and Hevesy (1942) introduced sodium chloride with added radioactive sodium into the blood of rabbits, dogs and cats and discovered that the whole of the sodium in the erythrocytes is exchanged for plasma sodium in 15 min in the rabbit, in 40 min in the dog and in 5-6 hr in the cat. This can be seen very well from the change in the distribution coefficient of the radioactive sodium between the erythrocytes and plasma with time (Table 61 and Fig. 86).

The maximum value of the distribution coefficient of the radioactive sodium is 11.0 for the rabbit, 15.8 for the dog and about 35 for the cat. This means that there is complete exchange of the erythrocyte sodium for plasma

TABLE 61. THE DISTRIBUTION OF RADIOACTIVE SODIUM IN RABBIT AND DOG BLOOD  
(from Hahn and Hevesy, 1942)

Subject	Time (min)	$\frac{^{24}\text{Na in 1 g of erythrocytes}}{^{24}\text{Na in 1 g of plasma}} \times 100$
Rabbit	2.3	5.7
	15.1	11.0
	32.0	10.0
	61.0	10.0
	120.0	11.0
Dog	4.0	6.0
	8.3	9.0
	13.6	10.9
	27.9	11.4
	41.2	15.8
	65.5	15.7

sodium, because the ratio of the concentrations of the total sodium in the erythrocytes to the concentrations of the total sodium in the plasma has the same value in the blood of these animals.

Fenn and his colleagues (Dean, Noonan, Haege and Fenn, 1941; Noonan, Fenn and Haege, 1941a; Mullins, Fenn, Noonan and Haege, 1941; Fenn, Noonan, Mullins and Haege, 1941) made a detailed study by the method

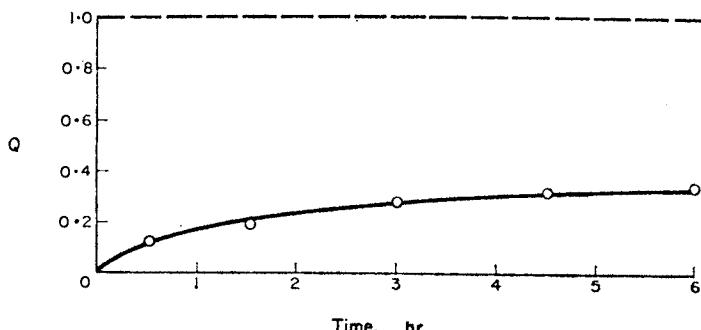


FIG. 86. The change in  $Q$  upon the distribution of radioactive sodium ( $^{24}\text{Na}$ ) between erythrocytes and plasma in cat blood with time (from Hahn and Hevesy, 1942).

of labelled atoms of the exchange of erythrocyte potassium in various animals and humans for plasma potassium upon the introduction of radioactive potassium into the blood. They discovered that the erythrocyte potassium of various animals is exchanged for plasma potassium at different rates. These authors also found that the rate of exchange of erythrocyte potassium is greater *in vivo* than *in vitro*. Table 62 shows the speed with which erythrocyte potassium is exchanged for plasma potassium. It shows that the speed of diffusion of potassium into the erythrocytes and back again is greatest in dogs and least in frogs. It is characteristic that dog erythrocytes contain very little potassium by comparison with the erythrocytes of other animals and humans.

In this work it was discovered that cat erythrocytes in *in vivo* and in *in vitro* experiments are easily penetrated by potassium and sodium alike. In conclusion these workers state that the widespread notion of the impermeability of the erythrocyte membrane for cations is incorrect: erythrocytes are permeable for both cations and anions. On this basis they come to the conclusion that it is impossible to explain the distribution of mineral ions between erythrocytes and plasma in terms of the classical membrane theory. Similar conclusions were reached by Hahn and Hevesy (1942), Hevesy (1942), and Kamen and Spiegelman, 1948).

Sheppard and his colleagues (Sheppard and Martin, 1948, 1949, 1950; Sheppard and Beyl, 1951; Sheppard, Martin and Beyl, 1951) developed a

TABLE 62. THE EXCHANGE OF INTRACELLULAR POTASSIUM FOR POTASSIUM  
OF THE BLOOD PLASMA IN HUMANS AND VARIOUS ANIMALS

(from Mullins, Fenn, Noonan and Haage, 1941)

Subject	Potassium concentration in cells (mm/l.)	Time for 30% exchange of potassium of erythrocytes ( <i>t</i> , in hr)	Permeability	Potassium circulation (% in 1 hr)
Dog	7.7	3.4	0.47	10.3
Cat	12.0	0.8	2.6	45.0
Rabbit	90	7.0	2.6	5.0
Human	85	8.2	2.4	4.3
Guinea pig	89	4.5	4.2	7.8
Rat	90	4.5	4.0	8.0
Frog	76	16 <sup>(1)</sup>	4.5	1.4

<sup>(1)</sup> In the case of frog erythrocytes 15 per cent exchange of cell potassium for plasma potassium has been taken, not 30 per cent.

TABLE 63. THE RATE OF EXCHANGE OF POTASSIUM AND SODIUM  
OF THE ERYTHROCYTES OF HUMANS AND VARIOUS ANIMALS

(from Sheppard, Martin and Beyl, 1951)

Subject	Concentration in cells (mm/l. of cells)		Rate of potassium exchange		Rate of sodium exchange	
	potassium	sodium	mm/l. of cells per hr	% exchange of cell potassium per hr	mm/l. of cells per hr	% exchange of cell sodium per hr
Human	91	10.8	1.65	1.8	1.5-0.1	14-1
Cow	25	70	0.95	3.8	11	16
Sheep	11.5	82	0.5-0.05	5.0-0.5	4	5
Dog	5.5	106	0.055	1.0	13	12.7 (~ 17 <sup>(a)</sup> )
Cat	6 <sup>(b)</sup>	104 <sup>(b)</sup>	—	—	~ 12 <sup>(c)</sup>	10-15 <sup>(c)</sup>
Rabbit	99 <sup>(b)</sup>	16 <sup>(b)</sup>	3.0	3.0 <sup>(d)</sup>	—	—
Rat	100 <sup>(b)</sup>	12 <sup>(b)</sup>	~ 6	~ 6 <sup>(d)</sup>	—	—

Note: from the data of <sup>(a)</sup> Cohn and Cohn, 1939; <sup>(b)</sup> Kerr, 1937; <sup>(c)</sup> Mullins, Fenn, Noonan and Haage, 1941; <sup>(d)</sup> Dean, Noonan, Haage and Fenn, 1941.

The table shows the rates of the quickly and slowly exchanging sodium fractions for humans and the corresponding potassium fractions for sheep.

method for determining the rate of exchange of potassium and sodium in the cells of whole blood for the same ions in the plasma by the introduction into isolated blood of radioactive sodium and potassium ions; the fall in the radioactivity of the plasma with time was then measured. Certain of the results obtained by this method are shown in Table 63.

Sheppard and Martin (1948) showed that radioactive potassium on introduction into heparinised dog blood rapidly disappears from the plasma and passes into the blood cells; the radioactivity of the plasma drops to half in 50 min. However, this rapid potassium exchange occurs owing to the leucocytes and the blood platelets. If these are removed from the blood by centrifugation, then the radioactivity of the blood plasma falls by a mere 1 per cent per hr. In another paper Sheppard and Martin (1949) found that the erythrocyte potassium of isolated human blood is completely exchanged for plasma potassium in 18 hr, the exchange in this case revealing a one-component exponential relation. They calculated that potassium exchange from human erythrocytes proceeds at a rate of  $10^{-13}$  mm of potassium per hr per cell, the rate for dog erythrocytes being  $4 \times 10^{-15}$  mm. The average rate of exchange of human erythrocyte potassium at  $38^\circ\text{C}$  is about 1.8 per cent of the cell potassium per hr. As the temperature is lowered, so the rate of exchange drops ( $Q_{10}$  is about 2.35). When the temperature is reduced below  $15^\circ\text{C}$  the rate of potassium flux into the erythrocytes is lower than the flux out of the erythrocytes into the plasma (Sheppard and Martin, 1950). Similar results are reported by Raker and his colleagues (Raker, Taylor, Weller and Hastings, 1950), who used the same method to study cell potassium exchange in diluted human blood. They found that at  $37^\circ\text{C}$  1.63 per cent of the cell potassium is exchanged for plasma potassium per hr. This represents 1.52 mm of potassium passing through the cell surface in both directions per litre of cells per hr or 190 potassium molecules passing through one square micron of erythrocyte surface per second.  $Q_{10}$  of this process is  $2.2 \pm 0.2$ . These authors also showed that change in the potassium concentration in the plasma from 2.02 to 74.4 mM/l. has practically no effect on the value of the constant of cell potassium exchange. Analogous results are reported in papers by other workers (Sheppard and Martin, 1950; Sheppard, Martin and Beyl, 1951; Solomon, 1952a, etc.). However, detailed investigations by a number of authors (Glynn, 1954, 1956, 1957a, b; Maizels, 1954 a, b; Harris, 1954; Shaw, 1955) show that the speed of penetration of potassium into erythrocytes and of release of sodium from the cells does not increase in direct proportion to the increase in the potassium (or sodium) concentration in the medium—the relation is more complicated.

Shaw (1955) in experiments with horse erythrocytes showed that the dependence of the potassium flux into the cells on the concentration of this ion in the medium can be described by an equation consisting of two components, one of which is linear and the other of the adsorptive type and corresponding to the Michaelis-Menten equation. As was shown by Glynn

(1956) in experiments with human erythrocytes, the presence of glucose in the cell suspension has no effect on the linear component but strongly increases the adsorptional component.

The permeability of human erythrocytes for potassium and sodium ions has been thoroughly studied with the aid of radioactive isotopes of potassium and sodium by Solomon and Gold (Solomon, 1952 a, b; Solomon and Gold, 1955; Gold and Solomon, 1955). According to Solomon (1952), potassium passes into the erythrocytes at a rate of 1.67, and sodium at a rate of 3.08 mequiv/l. of cells per hr. Solomon and Gold showed that both sodium and potassium ions are present in the cells, each in two fractions. The exchange of potassium and sodium from these fractions for the same ions from the medium is accomplished at different rates.

Tosteson and Robertson (1956) made a detailed study of the exchange of erythrocyte potassium for plasma potassium in the blood of white Peking ducks under various conditions of incubation of the blood. If the erythrocytes are suspended in dilute plasma, then after prolonged incubation a state of dynamic equilibrium is observed, in which the potassium and sodium concentrations in the cells do not change for several hours. In these conditions the flux of potassium ions into the erythrocytes and out of them is achieved at the same rate. The potassium flux through the cell envelope is strongly dependent on the composition of the gaseous phase in which the blood is incubated. In Table 64 we show data characterising this process. It can be seen that in an atmosphere of nitrogen the exchange of cell potassium for plasma potassium is achieved at appreciably greater speed than in an atmosphere of oxygen. In an atmosphere of oxygen all the cell potassium is exchanged for radioactive potassium from the medium in less than 24 hr, it being established that 4 per cent of the cell potassium is exchanged at a rate of 13.7 mM/l. of cells per hr and 96 per cent at a rate of 9.5 mM/l. of cells/hr. In an atmosphere of nitrogen all the potassium is exchanged at the same rate. In an atmosphere of oxygen the potassium flux at 30° is 7.4 mM/l. of cells/hr, and at 37° 8.1 ( $Q_{10} = 1.6$ ), whereas in an atmosphere of nitrogen at 30° the potassium flux is 8.8 and at 37° 26.0 mM/l. of cells/hr ( $Q_{10} = 4.2$ ). Increasing the pH of the medium from 7.02 to 7.82 in an atmosphere of oxygen leads to a lowering of the potassium ion flux into and out of the cells from 10 to 8.2 mM/l. of cells/hr, while increasing the pH from 7.06 to 7.75 in an atmosphere of nitrogen, on the contrary, increases the flux in both directions: into the erythrocytes from 15 to 35 and from the erythrocytes from 13 to 35 mM/l. of cells per hr.

Earlier, Tosteson (1954) found that the exchange of erythrocyte potassium in duck is achieved at a fifth of the rate in humans.

The permeability of the erythrocytes of mammalian animals and humans, and also of birds and reptiles, for potassium and sodium has been studied by chemical and radioactive indicator methods by many workers (Wilbrandt, 1940; Ting and Zirkle, 1940; Danowski, 1941; Harris, 1941; Maizels, 1943,

TABLE 64. THE MINERAL COMPOSITION OF DILUTE DUCK BLOOD. INCUBATION OF THE BLOOD FOR MANY HOURS IN AN ATMOSPHERE OF OXYGEN AND NITROGEN AT 37°C (from Tosteson and Robertson, 1956)

Composition of gaseous medium %	No. of ex- periments	Composition of erythrocytes			Concentration in dilute plasma (mM/l.)		Potassium flux (mM/l. of cells per hr)	
		concentration (mM/l. of cells)		% of water in cells	potassium	sodium		
		potassium	sodium					
95% O <sub>2</sub> + 5% CO <sub>2</sub>	10	112 ± 1.0	7.3 ± 0.97	59.3 ± 0.004	6.06 ± 0.44	141	8.0 ± 0.7	
95% N <sub>2</sub> + 5% CO <sub>2</sub>	21	106 ± 1.0	9.04 ± 0.73	64.5 ± 0.004	4.83 ± 0.18	145	28 ± 3.5	

1945, 1949, 1951, 1954, 1956, 1959; Ponder, 1947a, b, 1948, 1949, 1950, 1951; Flynn and Maizels, 1949; Harris and Maizels, 1951, 1952; Parpart and Green, 1950, 1951; Sheppard and Beyl, 1951; Sheppard and Stewart, 1952; Harris and Pranker, 1953a, b, 1955, 1957; Green and Parpart, 1953; Green and Wazeter, 1953; Grieg, Faulkner and Mayberry, 1953; Straub, 1953; Bernstein, 1954; Gardos, 1954; Thompson and Swan, 1959; Parpart and Hoffman, 1954; Hunter, Chalfin, Finamore and Sweetland, 1956; Clarkson, 1956; Glynn, 1956; Green, 1956; Flemming, 1956; Maizels and Remington, 1959a, b; Post, 1959; Bolingbroke and Maizels, 1959; Post, Merritt, Kinsolving and Albright, 1960 and many others). In these works attention is paid principally to the causes of the asymmetric distribution of potassium and sodium between erythrocytes and the medium surrounding them. In this connection studies were made of the effect on the distribution of potassium and sodium of a number of factors (high and low temperature, metabolic inhibitors, presence or absence in the medium of glucose, narcotics, hypo- and hyper-tonic salts solutions, change in pH, light energy, etc.). We shall return below to the discussion of the results obtained when considering the connection between metabolism and cell permeability.

It has been established that human erythrocytes are permeable for ions of rubidium, caesium and lithium. Rubidium passes into cells at about the same rate as potassium, caesium considerably more slowly (Solomon, 1952). If the sodium in the salt solution in which the human erythrocytes are being incubated is replaced by lithium, then sodium is released from the cells into the medium and lithium passes into the cells, the ratio of the lithium and sodium concentrations in the erythrocytes in time establishing itself at almost the same value as in the surrounding solution (Flynn and Maizels, 1949; Bolingbroke and Maizels, 1959; Maizels, 1959; Maizels and Remington, 1959a). Calcium chloride has a strongly depressing effect on the release of potassium and lithium from the erythrocytes and on the passage into the cells of sodium, whereas magnesium, strontium and barium chloride have no effect on these processes (Maizels, 1959).

*The permeability of leucocytes for ions.* Metabolic reactions in leucocytes occur at greater speed than in erythrocytes. The exchange of inorganic ions similarly takes place more rapidly. In Table 65 we show the potassium, sodium and water content in leucocytes of various kinds of animals. The concentration in leucocytes of ions (chloride, phosphate, bicarbonate) is approximately the same as in erythrocytes (Endres and Herget, 1929; Wilson and Manery, 1948, 1949). The mineral composition of the blood platelets is approximately the same as that of the leucocytes.

Wilson and Manery (1948) found that when rabbit erythrocytes are placed in Ringer's solution, in which the chloride concentration varies within the limits of 25 to 160 mequiv/l. (an equivalent amount of sodium chloride is replaced by sodium sulphate), the intracellular chloride concentration changes corresponding to the concentration of this ion in the medium but always

remains considerably lower than the external concentration. In these conditions, the sodium and potassium concentrations in leucocytes vary within limits of  $\pm 20$  per cent, but the sum of these cations remains constant. After the leucocytes have been left for 45 min at 37° in Ringer's solution containing 115 mequiv of chloride ions per l., there was found in them 80 per cent

TABLE 65. THE AMOUNTS OF POTASSIUM, SODIUM AND WATER IN THE LEUCOCYTES OF CERTAIN ANIMALS (from Hempling, 1954)

Subject	Potassium	Sodium	% water	Author
	mequiv/kg of cell water			
Dog fish ( <i>Mustelus canis</i> )	133.6 ± 10.6	88.6 ± 21.9	78.2	Hempling (1952)
Rabbit	105.1 ± 6.1	67.5 ± 11.1	79.0 ± 1.14	Hempling (1953)
Rabbit	106 ± 10	79.5 ± 7.8	79.25 ± 0.65	Wilson and Manery (1949)
Horse	23	113	84.57	Endres and Herget (1929)

water, 80–90 mequiv of chloride ions, 65–100 mequiv of sodium and 75 to 100 mequiv of potassium per l. of intracellular water. In another paper these writers (Wilson and Manery, 1949) showed that in sucrose solutions rabbit leucocytes quickly lose 80 per cent of their chloride ions and 50 per cent of their potassium ions.

In ammonium chloride solutions leucocytes lose 90 per cent of their potassium and 80 per cent of their sodium. In 75 min 90 per cent of the intracellular sodium is exchanged for sodium from the medium labelled with radioactive sodium.

A very high rate of exchange of intracellular potassium in the case of dog leucocytes and blood platelets for plasma potassium was discovered by Sheppard and his colleagues using radioactive potassium (Sheppard and Martin, 1948, 1949; Sheppard, Martin and Beyl, 1951).

Hempling (1953, 1954) studied the permeability of polymorphonuclear leucocytes of rabbits for potassium and sodium. In an exudative liquid with a potassium concentration in the medium of 2.0 mequiv/l. at 37° the leucocytes gradually lose potassium and absorb sodium in the same amount. In these conditions the potassium loss amounts to 5.76 per cent per hr of the total in the cells. A similar quantity of sodium passes into the cells. Raising the temperature to 36° and adding glucose to the medium leads to diffusion of the cations against the concentration gradient: the potassium again passes into the cells and the sodium is released from them into the medium. However, Elsbach and Schwartz (1959) in experiments on the same subject

and in analogous conditions found no equivalent exchange of potassium for sodium.

Butyl alcohol has the effect of raising the permeability of leucocytes for cations; the cells swell (Wilson, 1954).

Fleischmann (1929) came to the conclusion that calcium and barium ions do not penetrate horse leucocytes. He reached this conclusion on the basis of the fact that these ions do not depress the rate of breathing of intact cells, whereas there is found to be a considerable depressive effect on their respiration when the cells are damaged by freezing and thawing.

*The permeability of muscle fibres for cations.* Till recently it was thought that resting muscle fibres were impermeable for all anions and for sodium, lithium and the alkaline earth metals of the cations, being permeable only for the ions of hydrogen, potassium, rubidium, caesium and ammonium (Mond and Amson, 1928; Netter, 1928; Mond, 1930; Mond and Netter, 1930, 1932; Höber, 1932a, 1945, etc.). As we shall see below, these ideas were not confirmed.

Fenn and Cobb (1934-5) in experiments with isolated frog muscles made a detailed study of the distribution of potassium between muscle and medium in various experimental conditions. They showed that the amount of potassium in the muscle depends on its concentration in the medium and on the pH of the latter. An increase in the concentration of hydrogen ions in the medium leads to release of potassium from the muscle.

In another paper Fenn and Cobb (1935-6) described the absorption of potassium by isolated frog muscles immersed in the blood plasma with an elevated pressure of carbon dioxide or with an increase of the normal potassium content. The passage of potassium ions into muscle fibres from solutions containing a higher concentration of potassium was studied in detail by Boyle and Conway (1941). In these conditions potassium ions pass into the muscle fibres together with the chloride ions.

Fenn, Haege, Sheridan and Flick (1944) studied the penetration of ammonium ions into the muscles of frogs and other animals. They showed that isolated frog muscles absorb ammonium ions out of Ringer's solution in which the relevant part of the sodium chloride has been replaced by ammonium chloride. The ammonium concentration in the muscle after 5 hr at 5° reaches the concentration in the medium and after 4-5 days is already 50 per cent greater than in the surrounding liquid. The authors also showed that muscles in Ringer's solution containing ammonium chloride release potassium. The same is observed in experiments with kidney. However, the addition of ammonium chloride to the Ringer's solution has no effect on the release of potassium by the liver and the stomach muscles.

In muscle experiments tissue potassium is exchanged for ammonium, but not in equivalent amounts; in 5 hr muscle releases 6 mequiv/kg and absorbs 16.5 mequiv/kg of ammonium. Ammonium was also found to cause release of potassium from muscle in experiments where radioactive potassium was

employed. The introduction of ammonium chloride into cat blood leads to an increase in the blood potassium, which also points to release of potassium from the cells in exchange for the ammonium which goes into them. Potassium loss and ammonium absorption are very little dependent on the pH of the solution. When ammonium is absorbed muscle is enriched in chloride and loses part of its sodium. Thus, the chloride content in muscle is increased from 42.2 to 48 mequiv/kg, while the sodium content drops from 54 to 47.9 mequiv/kg by comparison with muscle which was kept for the same length of time in Ringer's solution without ammonium chloride. The speed of penetration of ammonium into muscles increases with increase in temperature and decreases with increase of the potassium, sodium, calcium and magnesium in the solution.

As has already been noted above, according to the classical membrane theory sodium ions do not penetrate muscle fibres, the whole of the muscle sodium being concentrated in the extracellular spaces. However, this notion of the extracellular localisation of sodium in muscle tissue is contradicted by numerous analyses of the mineral composition of muscles and their bathing liquids. Thus, the distribution of sodium between muscles and medium is essentially different from the distribution of chloride. In human blood plasma the ratio  $\text{Na}/\text{Cl} = 1.37$ , while in muscle it is 1.76. In frog plasma and muscle the corresponding values of the ratio are 1.47 and 2.14 (Fenn, Cobb and Marsh, 1934-5). The excess of sodium over chloride in muscle is unintelligible, as Fenn (1936) observes, if it is assumed that chloride and sodium are present only in the extracellular spaces. Fenn, Cobb and Marsh (1934-5) found for frog muscle that the sodium extracellular space was 24.5 per cent and the chloride space 14.5 per cent of the muscle volume. It is interesting that, when the muscles were left in Ringer's solution, the "sodium" space increased from 24.5 to 36 per cent and the "chloride" space from 14.5 to 31 per cent.

The majority of the proponents of the classical membrane theory admitted the penetration of sodium into muscle fibres only in the event of damage or excitation. However, there is to-day abundant proof that sodium normally passes unconditionally into muscle fibres, as into other live cells. Thus, Wu and Yang (1931) introduced a solution of sodium chloride intravenously into rabbits and found that the sodium penetrated the muscle fibre. Kaplanskii

TABLE 66. THE CONCENTRATION OF SODIUM IN THE BLOOD AND MUSCLE TISSUE OF CARP WHEN KEPT FOR 70 DAYS IN 1.5 per cent SOLUTION OF SODIUM CHLORIDE (in mg per cent, average data from many experiments) (from Kaplanskii and Boldyreva, 1934)

Tissue	Control	Experimental
Blood	123	129
Muscles	40	111.4

and Boldyreva (1933, 1934) showed that, when fish are kept in a medium with increased salts concentration, there is an accumulation of cations in the muscles without any change in their concentration in the blood. When carp were kept for one month in a 1·5 per cent solution of sodium chloride the amount of the cation of this salt in the muscles increased from 51·5 to 83·4 mg per cent, while the concentration in the blood remained the same as in the control fish. Carp kept under the same conditions for 70 days yielded the data shown in Table 66.

Similar results were obtained by Mond and Netter (1932). When the fish are placed in normal conditions, the sodium content in the muscles drops and quickly reaches its former level.

Kaplanskii and Boldyreva come to the conclusion that sodium on penetrating the muscle fibres forms weakly dissociated compounds with the proteins.

The penetration of muscle fibres by sodium is also indicated by the experiments of Steinbach (1940a, b, 1953). On immersing frog muscles into Ringer's solution without potassium he observed a gradual release of this cation from the muscles and the entry of an almost equivalent amount of sodium (Table 67).

TABLE 67. THE CONCENTRATION OF POTASSIUM AND SODIUM IN FROG SARTORIUS MUSCLES WHEN IMMERSED IN POTASSIUM-FREE RINGER'S SOLUTION (from Steinbach, 1940a)

Length of experiment (hr)	Potassium concentration (in mm/kg of tissue)	Sodium concentration (in mm/kg of tissue)
0	84·6 <sup>(1)</sup>	23·9 <sup>(1)</sup>
2	71·0	28
17	34·5	65
24	27·3	70
27	28·7	—

<sup>(1)</sup> From Conway and Boyle (1939).

A detailed analysis of the permeability of muscle fibres for ions of potassium, sodium etc. became possible when radioactive isotopes were applied to this work. The foundations were laid by Hevesy and Fenn and their colleagues (Hahn, Hevesy and Rebbe, 1939a, b; Hahn and Hevesy, 1941a, b; Hevesy and Hahn, 1941; Heppel, 1939, 1940a; Noonan, Fenn and Haege, 1941a; Fenn, Noonan, Mullins and Haege, 1941).

Heppel (1939) showed that if rats are fed for prolonged periods with food containing little potassium, then the amount of this cation in the serum is seen to fall from 7 mm to 2·6 mm and in the muscle from 110 to 64 mm/kg of tissue. In this time, the amount of sodium in the muscle increases threefold (from 18 to 54 mm/kg). Heppel (1940a) introduced intraperitoneally into

rats, the sodium content of whose muscles had been raised above the normal level, radioactive sodium (in the form of sodium chloride) and used the distribution of this isotope between muscles and plasma to determine the exchange of intramuscular for plasma sodium. It turned out that the whole of the muscle sodium was replaced by plasma sodium in less than 60 min. This shows that sodium ions penetrate the muscle fibres of mammals very quickly.

The penetration of radioactive sodium into the muscle fibres and also into the cells of other organs of dogs and rats follows from the results of Manery and Bale (1941).

In Table 68, which was assembled by Hodgkin from the results of many authors, we show the speed of penetration of sodium, potassium and chloride ions into the muscle fibres of isolated frog skeletal muscles in the resting

TABLE 68. THE ION FLUX INTO THE FIBRES OF ISOLATED RESTING FROG MUSCLES  
(in pm/cm<sup>2</sup> of fibre surface per sec) (from Hodgkin, 1951)

Ions	Approx ion concentration		Ion flux		Preparation	Author
	Med- ium	Muscle fibre	Into fibre	From fibre to medium		
Na <sup>+</sup>	115	25	—	14	Sartorius muscle	Levi and Ussing (1948)
	110	22	13	16	Sartorius muscle	Harris and Burn (1949)
	120	15	—	5-10	Sartorius muscle	Keynes (1949)
	120	15	—	5	Belly muscle	Keynes (1949)
	120	—	12	—	Long extensor of IV finger	Keynes (1951 b)
K <sup>+</sup>	2-5	125	6	—	Sartorius muscle	Noonan, Fenn and Haege (1941 a)
	3-4	125	27	—	All muscles of hind paw	Noonan, Fenn and Haege (1941 a)
	2-0	125	2	—	Sartorius muscle	Harris and Burn (1949)
	6-0	125	5	7	Sartorius muscle	Harris and Burn (1949)
	2-5	125	—	20	Sartorius muscle	Keynes (1949)
	2-5	125	10	10	Belly muscle	Keynes (1949)
	2-5	110	5	6	Long extensor of IV finger	Keynes (1951 b)
Cl <sup>-</sup>	5	125	6	8	Long extensor of IV finger	Keynes (1951 b)
	115	6	—	11	Sartorius muscle	Levi and Ussing (1948)

state. These data were obtained basically by means of radioactive isotopes. In calculation account was taken of the fact that 82 per cent of the volume of muscle fibre is occupied by water, and that the diameter of a fibre is  $80 \mu$ .

Keynes (1954), using the method of radioactive isotopes, studied the exchange of potassium and sodium from isolated frog muscles (sartorius, peritoneal and long abductor muscles of the IVth digit) for the sodium and potassium of Ringer's solution. In normal Ringer's solution (potassium concentration 2.5 mm) the potassium from the surrounding medium passes into the digital muscle at a rate of 4.1 pm (picomoles) per  $\text{cm}^2$  of muscle fibre surface per sec, the displacement of potassium from the muscle into the medium proceeding at a somewhat higher rate (4.9 pm). If the potassium concentration in the medium is doubled, the rate of potassium exchange is also doubled. The potassium flux into sartorius muscle in normal Ringer's solution is 10 pm/ $\text{cm}^2$  sec, the sodium flux out of the muscle fibres 5.4 pm/ $\text{cm}^2$  sec. Keynes found this quantity to be 13 pm/ $\text{cm}^2$  sec according to the data of Levi and Ussing (1948) and about 10 pm/ $\text{cm}^2$  sec from the data of Harris and Burn (1949) and Harris (1950). In this work Keynes also showed that the rate of exchange of muscle fibre sodium for sodium of the Ringer's solution increases about 2.5 times as the potassium concentration in the solution is raised from nil to 10 mm.

In another paper (Keynes and Swan, 1959a), when frog sartorius muscle was incubated in normal Ringer's solution, the sodium flux into the muscle fibres was found to be 4.2 pm/ $\text{cm}^2$  sec. If the sodium in the Ringer's solution was replaced by lithium or choline the sodium flux out of the muscle fibres was approximately halved by comparison with the flux in normal Ringer's solution. If the sodium concentration in the medium is changed, as these authors established, the sodium flux out of the muscle fibres changes not directly proportionally to the concentration in the external medium: the relation is of the adsorptional type obeying the Michaelis-Menten law.

According to Johnson (1955) the sodium flux out of frog sartorius muscles into Ringer's solution is 15 pm/ $\text{cm}^2$  sec. A similar value for the sodium flux in the case of this muscle was found by the present writer (Troshin, 1957). According to these measurements (Troshin, 1960a), the exchange of potassium of frog sartorius muscle fibres for potassium of Ringer's solution is accomplished at a rate of 10-16.4 pm/ $\text{cm}^2$  sec.

Harris and Burn (1949) showed that, in studying the exchange of muscle fibre potassium for potassium from the external medium, it is necessary to take into account the diffusional gradient in the extracellular spaces, which it is hard to do if one is dealing with muscles of differing weight. However, Abbott (1952) showed that muscles of different weights, such as sartorius muscles, which weigh about 100 mg, and the long abductor muscle of the IVth digit, which weighs 4 mg in all, in the same time (2.5-3 hr) exchange the same percentage of their potassium for potassium of the Ringer's solution. This shows that the rate of exchange of intercellular potassium (and sodium

likewise) is very high compared with the exchange of intracellular potassium and sodium (see Keynes, 1954; Troshin, 1957, 1960a).

In a recent paper Hodgkin and Horowicz (1959) studied the speed of movement of sodium and potassium into and out of isolated muscle fibres in the resting and active states. It was found by activational analysis that isolated muscle fibres (*m. semitendinosus*) of frogs (*R. temporaria*) had at the end of the experiment a potassium concentration of  $137 \pm 6$  mM/kg of intracellular water and a sodium concentration of  $26 \pm 3$  mM/kg of water. However, in the fibres of recently isolated muscles the potassium concentration was 139 mM/kg of water. The mean value of the sodium flux into the fibres was  $3.5 \text{ pm/cm}^2\text{sec}$ , and of the potassium flux  $5.4 \text{ pm/cm}^2\text{sec}$ ; the flux of sodium ions in the reverse direction was about  $3.5$  and of potassium ions  $8.8 \text{ pm/cm}^2\text{sec}$ . On the basis of the results obtained the authors concluded that the release of radioactive potassium and sodium from the muscle fibre into the non-radioactive medium obeys a simple exponential law, and that there is only one barrier for the free diffusion of these ions—the protoplasmic membrane of the fibre. These conclusions do not agree with the results of other workers (Harris, 1950, 1952, 1953, 1957; Edwards and Harris, 1957; Harris and Sjodin, 1959; Carey and Conway, 1954; Conway and Carey, 1955; Troshin, 1957, 1960a, and others). It is possible that these divergences are due to the fact that their experimental conditions did not allow Hodgkin and Horowicz to take into account the rate of loss by the fibres of labelled potassium and sodium in the first 3–5 min of the experiment. We shall return to the discussion of this problem in the following chapter.

As has already been noted above, according to the classical membrane theory, lithium ions, like sodium ions, cannot penetrate resting cells because of their high degree of solvation. However, the proposition turned out to be incorrect: both sodium and lithium penetrate cells. When the sodium ions in Ringer's solution, in which isolated frog muscles have been immersed, are partially or completely replaced by lithium ions, the result is that the lithium ions enter the muscle fibres in exchange for sodium ions (Keynes and Swan, 1959b; Briner *et al.*, 1959; Simon *et al.*, 1959; Muller and Simon, 1960; Troshin, 1960b). It has been shown that lithium ions penetrate frog sartorius muscle fibres at a considerably slower rate than sodium ions ( $1.2 \text{ pm/cm}^2\text{sec}$ , Keynes and Swan, 1959b), and that lithium is not able to cause the release of all the sodium from the fibres of these muscles (Troshin, 1960c).

In contrast to lithium ions, rubidium and caesium ions when added to Ringer's solution or used to replace potassium ions in this solution penetrate the muscle fibres and cause the release of potassium ions (Lubin and Schneider, 1957; Briner *et al.*, 1959; Muller and Simon, 1960 and a number of others).

Lubin and Schneider (1957) showed that rubidium and caesium ions can almost completely displace potassium from frog sartorius muscle fibres. This process, however, is achieved very slowly, the sodium concentration in the

muscle fibres remaining substantially unchanged the while. It was also shown that the rate of movement of caesium ions into and out of the muscle fibre is less than that of rubidium ions and considerably less than potassium ions. The same results were found by other writers as well (Mullins, 1959 and others). In Table 69, compiled from the data of Sjodin (1959), we show data characterising the permeability of muscle fibres for potassium, rubidium and caesium ions.

The permeability of the fibres of mammalian skeletal muscles for ions of the alkali metals (sodium, potassium and rubidium) does not differ in principle from the permeability for these cations of the muscle fibres of the skeletal muscles of amphibians. This conclusion can be reached from the work

TABLE 69. FLUXES OF IONS OF POTASSIUM, RUBIDIUM AND CAESIUM INTO THE FIBRES OF ISOLATED FROG MUSCLES AT ROOM TEMPERATURE (from Sjodin, 1959)

Concentration of ions in Ringer's solution (gmm/1)	Ion flux into the muscle fibres	
	in $\mu\text{M}/\text{g}$ of muscle fibre/hr	in $\text{pm}/\text{cm}^2\text{sec}^{(2)}$
Potassium - 2.0	5.9 <sup>(1)</sup>	3.1
2.5	7.3	3.8
4.0	11.3 <sup>(1)</sup>	5.9
5.0	13.9	7.2
8.0	19.7 <sup>(1)</sup>	10.2
10.0	22.0	11.8
15.0	30.0	15.6
20.0	39.0	20.3
40.0	61.1	31.8
Rubidium - 0.6	0.877	0.5
2.5	3.6	1.9
10.0	8.0	4.2
100.0	16.0	8.3
Caesium - 0.6	0.229	0.1
2.5	-	-
12.5	4.3	2.2
113.0	10.9	5.7

<sup>(1)</sup> From Harris (1957a).

<sup>(2)</sup> Calculated by myself from the data of the second column on the assumption that the surface of 1 g of muscle fibres is  $530 \text{ cm}^2$  (Harris, 1957a).

of a number of authors (Creese, 1951, 1954; Flückiger and Verzár, 1954; Grossweiler *et al.*, 1954a, b; McLennan, 1955; Conn and Wood, 1957; Menozzi *et al.*, 1959; Zierler, 1960, etc.). It should be noted that the exchange of intracellular ions of potassium and sodium for the same ions from the medium is achieved at a considerably higher rate than is observed in cold-blooded animals. In Tables 70 and 71 we show by way of example data from

TABLE 70. THE DISTRIBUTION OF POTASSIUM AND SODIUM IN THE DIAPHRAGM AND BLOOD OF RATS (from Creese, 1954)

Subject	Water content g/kg of tissue	Specific gravity	mM/kg of wet weight of tissue	
			sodium	potassium
Diaphragm ( <i>in vivo</i> )	770 ± 17 (20)	—	42.0 ± 5 (20)	86.2 ± 6 (35)
Diaphragm in Krebs' solution	786 ± 10 (20)	1.055	70.0 ± 11 (20)	80.0 ± 6 (20)
Blood	813 ± 8 (10)	1.053	79.4 ± 7 (6)	41.5 ± 2 (6)
Plasma	928 ± 7 (6)	1.022	138 ± 8 (10)	4.6 ± 0.2 (7)

The figures in round brackets indicate the number of analyses.

TABLE 71. INDICES OF THE EXCHANGE OF POTASSIUM AND SODIUM OF RAT DIAPHRAGM FOR LABELLED POTASSIUM AND SODIUM IONS OF KREBS' SOLUTION (from Creese, 1954)

	<i>in vivo</i>		<i>in vitro</i>	
	Na <sup>+</sup>	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>
1. Intracellular concentration (mequiv/kg of cell water) <sup>(1)</sup>	18	158	60	149
2. Ratio of intracellular/extracellular concentration	0.13	34	0.41	25
3. Diffusion constant in intercellular space (cm <sup>2</sup> /sec × 10 <sup>-6</sup> )	—	—	4.2	5.2
4. Half time of exchange, T <sub>1/2</sub> (min)	—	—	11	36
5. Ion flux (pM/cm <sup>2</sup> sec)	—	—	28	21

<sup>(1)</sup> Intercellular space, determined in respect of inulin, is 26.4 ml/100 g of muscle ± 3.1 ml (average of 24 analyses).

the paper by Creese (1954) which show the amount of sodium and potassium in the muscle fibres of rat diaphragms and the rate of exchange of these ions.

It is interesting to note that isolated muscles of warm-blooded animals, when immersed in Ringer's or Krebs' solution, release potassium and absorb sodium to a greater extent than cold-blooded animals. Creese asserts that the exchange of potassium and sodium from rat diaphragms for the same ions from Krebs' solution obeys a simple exponential law. However, this is contradicted by more recent results obtained by a number of workers (McLennan, 1955; Conn and Wood, 1957; Menozzi *et al.*, 1959).

Zierler (1960), experimenting with *m. extensor digitorum longus* of male rats showed that the potassium efflux is 36 (at 37°) and 21 pM/cm<sup>2</sup>sec (at 26°). Insulin depresses the entry and exit of potassium from muscle. Calcium ions have the same effect (Flückiger and Verzár, 1954; Grossweiler *et al.*, 1954a, 1954b, etc.).

A number of workers have investigated the mineral ion content and exchange in mammalian heart muscle. Barclay *et al.* (1959) showed that the concentration of sodium and chloride ions in the auricles of rat hearts is significantly higher than in the ventricles. On perfusion of the heart with Ringer-Locke solution the heart muscle is enriched in these ions, the left and right ventricles absorbing sodium and chloride in the process. In these conditions, as was shown by Goodford (1959) in experiments with isolated rabbit hearts, the muscle fibres lose a considerable amount of potassium.

Humphrey (1959) showed that the muscle fibres of isolated rabbit hearts lose potassium into a potassium-free medium at 35° at a rate of 4.65 mequiv/kg/min, while the flux of potassium ions labelled with radioactive potassium into the muscle is equal to 4.54 mequiv/kg/min. At 20° the quantities are, respectively, 1.78 and 1.88 mequiv/kg/min. The temperature coefficient for potassium loss from the heart muscle is 2.2.

Rayner and Weatherall (1959) studied the exchange of potassium ions from the auricles of isolated rabbit hearts for the same ions from the medium labelled with radioactive potassium. The flux of potassium ions into the fibres of the uncontracted left auricle is 11.4 pm/cm<sup>2</sup>/sec, and the flux in the opposite direction 16.9 pm/cm<sup>2</sup>/sec. On spontaneous contraction of the auricle or stimulation by electric current of a quiescent auricle, the potassium ion fluxes rise. The same effect is produced by acetylcholine.

Some interesting experiments on potassium and sodium exchange by heart muscle of embryos and hatched chicks were made by Klein (1960). He found that in various periods of embryonic development the intracellular concentration of sodium is very high, subsequently falling quickly. Thus, in a two-day embryo it is 350–900 mM, in a three-day embryo 250–575 mM, while three days after hatching it falls to 50–75 mM. The external embryonic fluid contains 100–130 mM of sodium and 5–10 mM of potassium. Similar concentrations of sodium and potassium were found in the fluid of the amnion and allantois. The loss of potassium from the stomach of a seven-day embryo, as determined by means of radioactive potassium, is made up of two exponential components. One of these is fast—with a half time of 2 min, the other slow—with a half time of 41 min. In the later stages of embryonic development the rate of potassium ion flux into and out of the muscle fibres falls.

The sodium ion flux outwards, as determined by means of radioactive sodium, from the stomach muscle fibres of a twelve day embryo is characterised by three exponential components with half times of 3, 12 and 43 min respectively.

The permeability of muscle fibres has been established for certain bivalent cations too. Thus, Fenn and Haege (1942) immersed isolated frog muscles in Ringer's solution containing from 0 to 6 mM magnesium: they found that after 5 hr in these solutions the muscle lost a certain amount of magnesium if the concentration in the medium was less than 1.0 mM/l, and

absorbed it if the concentration was greater than this. It was found that the penetration of magnesium occurs partially with chloride. The entry of magnesium into muscle fibres is accompanied by the displacement of potassium, 1 mM of magnesium displacing 10 mM of potassium. The authors suppose that potassium and magnesium in muscle fibre are partially bound, probably with myosin, and can compete with one another for the same positions.

Krogh and his colleagues (Krogh and Lindberg, 1944; Krogh, Lindberg and Schmidt-Nielsen, 1944), experimenting with isolated frog hearts by chemical methods, showed that the muscle fibres of this organ are permeable for potassium, sodium and calcium, just as they are permeable for glucose and sucrose. Depending on the concentration in the medium, these substances can pass into or out of the muscle fibres. Kaplanskii and Boldyreva (1933), studying the regulation of mineral exchange in homosmotic fish, discovered that, when the calcium ion concentration in the medium is increased, the calcium concentration in the muscles of the latter is increased. Veshetko and Kozlovskii (1954) observed absorption of calcium by the muscles and other tissues upon the introduction of this cation into the blood of rabbits.

Woodward (1949), using radioactive calcium, found that the intracellular calcium of frog muscles is exchanged for the calcium of Ringer's solution. This exchange is enhanced by stimulation of the muscles.

The permeability of the muscle fibres of cold-blooded and warm-blooded animals for ions of the alkaline earth metals has been demonstrated both by chemical techniques and by the use of radioactive isotopes (Berwick, 1951; Harris, 1955; Mullins, 1959; Bianchi and Shanes, 1959; Frater, Simon and Shaw, 1959; May and Barnes, 1960, etc.).

The permeability of muscle fibres for bivalent cations is considerably lower than their permeability for univalent cations (Harris, 1955; Bianchi and Shanes, 1959; Mullins, 1959a). By way of illustration we show in Table 72

TABLE 72. CATION FLUX INTO MUSCLE FIBRES OF ISOLATED FROG SARTORIUS MUSCLE  
(from Mullins, 1959a)

Ions	External concentration of cation $C_o$ (mM)	Ion flux into fibres (mm/kg fibre water per hr)	Ion flux in mm/kg fibre water per hr/mm $C_o$	Relative permeability of fibre
Potassium	5	9.6	1.92	1.00
Rubidium	2.5	2.6	1.04	0.54
Caesium	2.5	0.54	0.21	0.11
Sodium	110	9.3	0.0845	0.043
Barium	0.025	0.0004	0.016	0.008
Calcium	1.80	0.009	0.005	0.0026
Radium	0.30	0.0015	0.005	0.0026

data on the permeability of frog sartorius muscle fibres for ions of the alkaline and alkaline earth metals. These data were obtained by Mullins (1959a) with radioactive isotopes. However, it should be noted that the very strong difference in the rate of penetration in a number of the cations may be exaggerated, because the flux of ions was determined for differing concentrations in the medium. We have already cited data above which indicate that the rate of penetration of ions into muscle fibres is not linearly related to the concentration of the ion in the surrounding solution (Keynes and Swan, 1959a; Sjodin, 1959). Further, Mullins did not in these experiments take into account the possibility of the cations being "bound" in the muscle sarcoplasm.

According to Bianchi and Shanes (1959), a considerable amount of the calcium in the sarcoplasm is found in the bound form. The calcium ion flux into frog muscle fibres is about  $0.094 \text{ pm}/\text{cm}^2\text{sec}$ .

The data cited above indicate that the muscle fibres of striated muscles are permeable for alkaline and alkaline earth cations.

*The permeability of muscle fibres for anions.* Mond and Amson (1928), on the basis of analysis of the perfusion liquid flowing off frog muscles, came to the conclusion that chloride, thiocyanate and bromide do not penetrate muscle fibres.

In spite of these assertions, there is a large amount of experimental material which shows that all anions and, in particular, phosphates and chlorides can penetrate live cells, including muscle fibres.

The presence of chloride in muscle fibres is proved by direct chemical analysis of individual muscle fibres. Heilbrunn and Hamilton (1942) analysed individual frog muscles and found up to 41.5 mequiv of chloride per kg of muscle fibre. According to these authors, only 20 per cent of the total chloride in these muscles is located in the intercellular spaces, 80 per cent being inside the muscle fibres. According to their calculations, the equilibrium chloride concentration in the muscle fibre is 30 per cent of the concentration in the plasma. Similar data are given by Dean (1941a, b).

It has long been known that isolated muscles when immersed in Ringer's solution lose a certain amount of potassium and absorb chloride and sodium (Fenn, 1935, 1936; Stephenson, 1953a, b; Carey and Conway, 1954; Creese, 1954, etc.). Fenn (1935) placed frog muscles in Ringer's solution and after certain time intervals determined the chloride content. If there were originally 0.723 mequiv of chloride per 100 g of muscle, then after 5 hr in the Ringer's solution at 22° the muscles contained as much as 2.85 mequiv, and after 12 hr 3.01 mequiv of chloride.

The absorption by frog muscles of chloride, but in smaller quantities, also occurs when they are placed in the blood plasma of this animal (Fenn and Cobb, 1934-5).

In another paper Fenn, Cobb and Marsh (1934-5) made a detailed study of the penetration of chloride (together with sodium) into isolated frog

muscles upon immersion at room temperature into Ringer's solution with varying chloride content (part of the sodium chloride in the Ringer's solution was replaced by sodium nitrate). They found that at room temperature chloride and sodium penetrate the muscle but not in equal amounts. Thus, after 5 hr the chloride concentration in the muscles for all concentrations in the medium reached 31 per cent of the latter, while the sodium concentration was 33 per cent. Fenn and his colleagues were inclined to explain their results as showing that chloride penetrates only the extracellular spaces, which increase proportionately to the volume of muscle in this period from 14·7 to 31 per cent, and do not penetrate the muscle fibres, while sodium penetrates partially and replaces a certain amount of potassium. Subsequently, Fenn and Haege (1942) rejected this explanation of the phenomenon and concluded that chloride also penetrates the muscle fibres.

Steinbach (1937) studied the permeability of the retractor muscles of the holothurian *Thyone briareus* for potassium and chloride. The extracellular space of these muscles amounts to 17·8 per cent, the myofibrils 52·6 per cent and the sarcoplasm 29·6 per cent of the muscle volume. Normally the muscle contains 20·4 mm of chloride per 100 g of fresh tissue. On placing it in salt solutions containing various chloride concentrations (the different chloride concentrations were achieved by the dilution of sea water with isotonic sugar solution), the author observed absorption of chloride or emission of it by the muscle depending on the concentration in the surrounding solution. For all the tested chloride concentrations in the medium Steinbach found there to be in the muscle 43 per cent of the concentration in the solution. If the chloride did not penetrate the muscle fibres but was entirely contained in the extracellular spaces, then one would have expected to find in the muscle 17·8 per cent of the amount in the medium (because the extracellular space occupies 17·8 per cent of the muscle volume).

The permeability of muscle fibres for chloride was later studied in greater detail by Boyle and Conway (1941), Wilde (1945) and others. They also came to the conclusion that chloride penetrates inside muscle fibres and freely passes out of it entirely upon immersion of the muscle into a solution not containing this anion. According to Boyle and Conway (1941), muscle fibres are permeable not only for chloride ions, but also for  $\text{OH}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$  and  $\text{NO}_3^-$ , but are impermeable to  $\text{CH}_3\text{COO}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{HPO}_4^{2-}$ . Harris (1952, 1953a, 1958) also asserts that muscle fibres are not permeable for sulphate and orthophosphate.

The permeability for chloride ions of the fibres of amphibian skeletal muscles has also been studied by a number of workers (Shuck, 1950; Carey and Conway, 1954; Edwards *et al.*, 1957; Simon *et al.*, 1957, 1959; Harris, 1958; Frater *et al.*, 1959; Hodgkin and Horowicz, 1959 and others).

The penetration of chloride ions into the muscle fibres of rat diaphragms was shown in a paper by Conway and Fitzgerald (1942) and into the muscle fibres of the crab *Carcinus maenas* by Shaw (J. Shaw, 1955a, b).

The present writer (Troshin, 1953a) studied the distribution of chloride ions between isolated frog gastrocnemius muscles and a medium containing various concentrations of this ion.

The chloride concentration in the medium was varied as follows. Two solutions were prepared: Ringer's solution and the same solution in which the sodium, potassium and calcium chlorides had been replaced by the nitrates of these cations taken in the same molar concentrations. By mixing equal amounts of these solutions a third was prepared—Ringer's solution with half the chloride content.

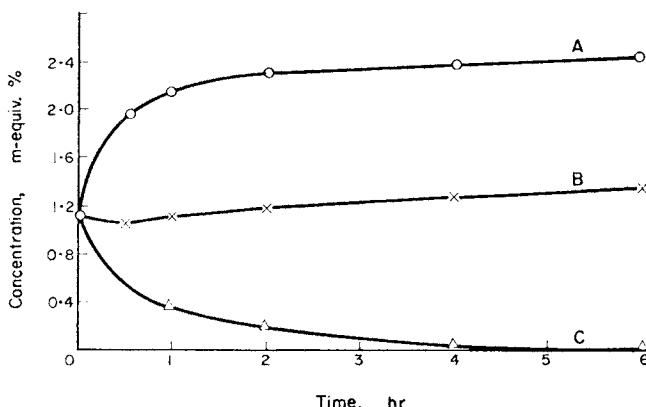


FIG. 87. The change in the chloride ion concentration in frog gastrocnemius muscles with time.

A—in Ringer's solution; B—in Ringer's solution with half chloride content;  
C—in chloride-free Ringer's solution.

The experiments were conducted at room temperature (18–20°) on the muscles of autumn frogs. The results are portrayed in Fig. 87.

According to these analyses, frog gastrocnemius muscles immediately after removal from the organism contain 1.13 mequiv per cent of chloride (mean of 12 analyses). Figures close to this for frog skeletal muscles are given in their several papers by Katz (1896), Urano (1908), Hill and Kupalov (1930), Fenn (1936), Boyle and Conway (1941) and others. Thus, according to Fenn (1936), frog sartorius muscle contains 1.09 mequiv per cent of chloride.

If the muscles are placed in normal Ringer's solution containing 11.17 mequiv per cent of chloride ions, then already after 1 hr the concentration of the latter in the muscles has almost doubled (from 1.13 to 2.13 mequiv per cent), thereafter increasing only slowly and finally reaching 2.45 mequiv per cent after 6 hr.

Frog gastrocnemius muscles placed in Ringer's solution with half the chloride concentration (0.58 mequiv per cent) at first lose a small amount of this ion and then begin to absorb it. After one hour the chloride concentration in the muscles reaches the normal level and after 6 hr it increases to 1.38 mequiv per

cent (an increase over the normal of 0·25 mequiv per cent; see Fig. 87). In contradistinction, muscles immersed in chloride-free Ringer's solution lose the great mass of this ion in the first 2 hr, after which the process slows down, so that it is only after 6 hr that almost all the chloride has come out of the muscle tissue. The results of the analyses of this series of experiments are in agreement with the data of many authors, who also established that there is rapid loss of chloride from muscles and nerves immersed in a medium free of this ion (Fenn, Cobb and Marsh, 1934/5; Fenn, Cobb, Hegnauer and Marsh, 1934/5; Steinbach, 1940b, 1941; Boyle and Conway, 1941 and others).

Hodgkin and Horowicz (1959) in experiments with single frog muscle fibres discovered that chloride ions penetrate these muscles at a greater rate than potassium ions. The same conclusions were reached by Adrian (1956).

Harris (1958) studied the permeability of frog muscle fibres for the anions  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{I}^-$ ,  $\text{ClO}_4^-$ , and  $\text{CNS}^-$ . It was established that chloride ions can penetrate muscle fibres or pass out of them in exchange for the other above mentioned ions, in exchange for the same chloride ions (in experiments with radioactive chloride) or together with a cation (potassium, sodium, etc.). The rate of emergence of chloride ions out of muscles previously enriched in this anion by immersion for 1 hr in a 120 mM solution of potassium chloride or the rate of exchange of the labelled chloride anions in the muscles for chloride anions from the Ringer's solution is retarded if part of the chloride ions in the latter is replaced by other anions: in order of effectiveness in depressing the release of chloride ions the following series is obtained:  $\text{CNS}^-$  and  $\text{ClO}_4^-$  (equal first),  $\text{I}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ . This series corresponds to the lyotropic series of these anions.

Edwards, Harris and Nishie (1957) showed that the replacement in salt solutions of chloride ions by  $\text{NO}_3^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$  and  $\text{CNS}^-$  has no noticeable effect on the rate of exchange of sodium and potassium in frog skeletal muscles for the same cations in the medium;  $\text{CNS}^-$  is more poisonous than the other anions, depressing the rate of flux of potassium into the muscle fibres. Simon *et al.* (1959) showed that the anions  $\text{Br}^-$  and  $\text{I}^-$  easily penetrate the skeletal muscles of the toad *Bufo marinus* and can replace completely the chloride ions of the muscle fibres, while they do not noticeably affect the loss of potassium by the muscles. A number of writers (Johnson, 1955; Frater, Simon and Shaw, 1959; Zhinkin and Zavarzin, 1960 and others) have shown that skeletal muscle fibres are permeable for sulphate. According to Simon and his colleagues, sulphate on penetrating muscle fibres takes less water for its solution than sodium. The distribution of sulphate between muscle fibres and the medium is comparable with the distribution of lithium, magnesium, iodine and orthophosphate ions (see Briner, Simon and Shaw, 1958).

The basic mineral anion in muscle, as is well known, is the anion of phosphoric acid. According to the data of Fenn (1938a), frog muscles contain 16·0 mequiv/kg of fresh tissue of this anion.

Embden and Adler (1922) were the first to observe the emission of phosphoric acid ions from isolated frog muscles immersed in Ringer's solution. In time and after stimulation of the muscles phosphate was observed to be released in greater quantities than in the resting state. The authors connect this phenomenon with a change in the properties of the membrane. Undamaged muscle membranes, and also the membranes of resting muscles, are supposed to be completely incapable of passing this anion through themselves.

Fenn (1931) observed the release of phosphate from frog muscle immersed in isotonic sugar solution, while Harris (1953b) found the same for immersion in salt solution free of the phosphate anion. According to the determinations of this writer, frog sartorius muscle loses about  $62 \mu\text{M}$  of inorganic phosphorus (or about 2·5 per cent of the inorganic cell phosphorus) in the first 3 hr. The rate of phosphorus absorption into the muscle, as found with the aid of radioactive phosphorus, was significantly lower.

A detailed study of the release of phosphates from frog muscles was made by Il'yin and Tikhalskaya (1931). On the basis of numerous experiments they reached the following conclusions: (1) in the state of rest muscles are permeable for phosphoric acid ions; (2) after direct excitation of the muscle by electric current, the release of phosphate is increased; (3) increase in the release of phosphates above the normal is observed also after excitation of the muscles through the nerves.

The authors are inclined to explain the enhanced phosphate release on excitation not by an increase in the permeability of a membrane, but by the reformation of this anion during the period of activity of the muscles from organic phosphorus compounds. In proof of this the authors adduce much data.

In a discussion of the published data on the release of phosphate from muscle at rest and in a state of excitation, Fenn (1936) denies the possibility of the penetration of this anion, and also of other anions, into muscle fibres and of their release from them. He writes: "The admission even of slow loss of phosphate would present great difficulties for the membrane theory, because in this case potassium and phosphate could be slowly expended by muscle" (p. 464). He assumes that *in vivo* in the state of rest the muscle membrane is completely impermeable for phosphate.

However, this supposition of Fenn's turned out to be erroneous.

In Fig. 88, we show the accumulation of radioactive phosphorus and sodium in frog sartorius muscles with time. It can be seen in the figure that in the first 1–2 hr the concentration of radioactive phosphorus and sodium in the muscles reaches about 20 per cent of their concentration in the plasma. With time the distribution coefficient of the radioactive phosphorus slowly increases and towards 96 hr the concentration of labelled phosphorus becomes 2·4 times greater than in the medium. The right branch of the distribution curve of this anion characterises the process of its inclusion in organic com-

pounds. In contrast to the phosphorus, the distribution curve for the labelled sodium remains for the whole of this period at the level of 20 per cent. This indicates that the whole of the muscle sodium is exchanged for plasma sodium already in the first 1–2 hr.

Using the same method, Manery and Bale (1941) showed the penetration of phosphate into muscle fibres and into the cells of several other tissues of dogs, rats and rabbits. The penetration of radioactive phosphorus into muscle fibres and the cells of other organs of rats was observed also by Gaunt,

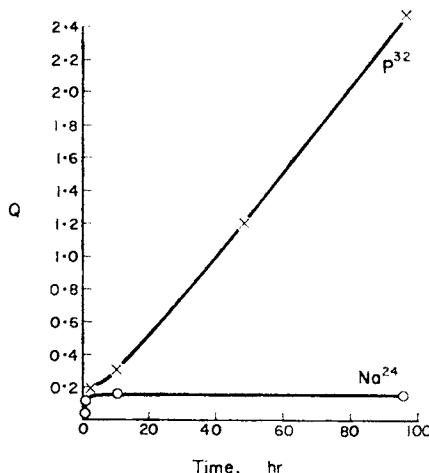


FIG. 88. The change in  $Q$  upon the distribution of radioactive sodium ( $^{24}Na$ ) and phosphorus ( $^{32}P$ ) between gastrocnemius muscles and blood plasma of frogs with time (from Hevesy and Rebbe, 1940).

Griffith and Irving (1941), Hevesy and Ottesen (1943) and others. The permeability of muscle fibres and of the cells of a wide variety of organs of fish and other organisms was demonstrated in the works of many authors, who developed a method of marking the animals with radioactive phosphorus and other radioactive elements (for a summary of the literature see Kirpichnikov *et al.*, 1958).

Furchtgott and Shorr (1943) studied the inclusion of mineral phosphate into inorganic phosphate, creatine phosphate and adenosine triphosphate of dog heart muscle in *in vitro* experiments in a medium containing  $Na_2H^{32}PO_4$ . According to them, at a temperature of  $37.5^\circ$  the mean rate of exchange of medium phosphorus for muscle fibre phosphorus is  $1.7 \mu\text{g}/\text{min/g}$  of tissue. This rate of exchange is much greater than the rate of inclusion of phosphorus in rabbit sartorius muscle fibres. Further, in experiments at  $2^\circ$  they showed that extracellular inorganic phosphate is exchanged directly with intracellular inorganic phosphate (without organic phosphate as intermediary).

According to their data, cellular inorganic phosphate is a direct donor of organic phosphorus compounds. They further point out that the concentration of intracellular labelled inorganic phosphate always remains lower than the concentration of labelled inorganic phosphate in the medium.

Kalckar *et al.* (1944) also showed that orthophosphate penetrates muscle fibres because of free diffusion. The same standpoint on this question was taken by Ennor and Rosenberg (1954).

Sacks and Altschuler (1942) were among the first to suggest the idea that the passage of mineral phosphorus into muscle fibres is connected with its inclusion in the cell membrane into organic phosphorus compounds. They reached this conclusion on the basis of the fact that, upon injection of  $\text{Na}_2\text{H}^{32}\text{PO}_4$  into frog or cat blood, the radioactive phosphorus is in the first place detected in organic phosphorus compounds, and only after that is it included in the fraction of muscle mineral phosphorus.

In subsequent papers Sacks (1944a, b) came to the conclusion that the external mineral phosphorus in the membrane of muscle fibre is included in glucose-6-monophosphate and in this form passes into the cell, after which it is included in other organic phosphorus compounds. Glucose too does not pass freely into muscle fibres. In the membrane, so they suppose, it is converted into glucose-6-monophosphate. In support of this they adduce the fact that in the presence of glucose in the medium the rate of penetration of phosphorus into muscle cells is enhanced. Sacks (1945) further adds that the addition of insulin increases the rate of formation of labelled glucose-6-monophosphate. This effect also occurs in the event of muscular work (Sacks, 1948). However, Tigyí (1959) showed that the rate of passage of phosphorus into contracted frog muscles, on the contrary, is depressed and not enhanced.

Causey and Harris (1951), using autoradiography, attempted to show that labelled mineral phosphorus from the medium is first of all included in the organic compounds of frog sartorius muscle fibre. These compounds are localised, as they showed, near the surface of the fibre, which, as these workers consider, confirmed Sacks' idea of the mechanism of the penetration of mineral phosphorus into muscle fibres.

Eichler and Schmeiser (1951) studied the absorption of ortho- and pyrophosphate by the muscle fibres of frog's hearts. The experiments were performed on isolated ventricles, through which was passed Ringer's solution containing labelled phosphate in various concentrations. The ventricle was excited by electrical impulses. It was found that at low phosphorus concentrations ( $0.184 \mu\text{g}/\text{ml}$ )  $0.008\text{--}0.048 \mu\text{g}/\text{g}$  of tissues was absorbed per minute. Increasing the phosphate concentration in the medium by more than 20 times leads to only an insignificant increase in the absorption of phosphorus by the muscle. Damage to the heart (change in acidity, osmotic pressure and exclusion of air) leads to an increase in the absorption of phosphate. The authors assert that orthophosphate, before passing into the muscle fibre, is converted

on its surface into hexose-6-phosphate and that the muscle fibre is impermeable for orthophosphate. However, the experiments with the exclusion of air indicate increased phosphorus absorption, whereas it should be reduced, because the process of hexose-phosphate formation requires an energy channel. The authors suppose that there occurs here some new, unknown phenomenon. According to their observations, pyrophosphate is absorbed more strongly than orthophosphate and terminal equilibrium is achieved more quickly in this case. They make this dependent on the activity of the enzymes on the surface of the muscle fibres.

Quite recently, the permeability of the muscle fibres of amphibians for orthophosphate was convincingly shown in papers by Briner *et al.* (1958) and Pisareva (1959, 1961). The results of these most interesting studies will be discussed in detail in the following chapter.

Thus, the assertion of the impermeability of muscle fibres for anions and their selective permeability for certain cations is not confirmed experimentally: muscle fibres are permeable for all mineral anions and cations.

#### *The permeability of smooth muscle fibres for ions.*

In the opinion of the supporters of the membrane theory, the fibres of smooth muscles have completely different properties in respect of their permeability for salts from the fibres of striated muscles (Gellhorn, 1929; Rubinshtein, 1947). This assertion, however, ties up but poorly with the general propositions of the membrane theory.

The muscle fibres of smooth muscles do not differ in principle in their mineral composition from the fibres of striated muscles. For comparison the relevant data for the frog are shown in Fig. 73.

It may be seen from these data that the differences between the concentrations of individual ions in the blood plasma and in the muscle fibres of smooth muscles are just as significant as they are in the case of the distribution of ions between striated muscles and plasma.

The difference in the mineral composition of smooth muscles fibres and the plasma can hardly have a different cause or different mechanism from the mechanism of the distribution of salts between the muscle fibres of striated muscles and the plasma. This complication, with which the membrane theory is confronted here too, is discussed by one of the upholders of the theory in his book (Gellhorn [1929]).

Meigs (1912, 1913, 1914, 1915) also supposed that the permeability of smooth muscles is different in principle from the permeability of striated muscles and other cells. The smooth muscles of the stomach also have completely different osmotic properties. In an isotonic solution of potassium chloride smooth muscles decrease in weight and in an isotonic solution of sodium chloride they swell, i.e., for a solution of the first electrolyte the opposite phenomenon occurs to that observed in experiments with striated muscles. It is possible to extract up to 50 per cent of the potassium from

the stomach muscles by immersing them in sucrose, the excitability of the muscles being unchanged in these conditions.

In experiments with the smooth muscle of the adductor of the shell of the molusc *Venus mercenaria* Meigs (1914) showed that it loses all its chloride in sucrose. Normally this muscle contains 0·32 per cent chloride and the blood 70 per cent. After 24 hr in sea water the muscle contains 0·84 per cent chloride, and after the same period in sea water containing doubled salts' concentrations the chloride concentration in the muscles reaches 1·68 per cent. In a 10 per cent solution of sodium chloride the volume of the muscle is not reduced and its excitability is unchanged. On the basis of these experiments the author comes to the conclusion that smooth muscle fibres do not have a semi-permeable membrane and do not behave like osmometers, in contradistinction to striated muscles.

Heymann (1925) investigated the permeability of the smooth muscles of frog's stomach for salts and sucrose and found that sucrose freely penetrates the muscle fibres, while potassium is released into the surrounding sugar solution. These muscles can give up up to 50 per cent of their potassium and 55 per cent of their calcium into an isotonic sugar solution without any change in their excitability. This author established that calcium chloride penetrates the muscle, though equality of its concentrations in the muscle and medium is not achieved.

Singh and Acharya (1957) in experiments with the stomach muscle of the frog *Rana tigrina* established that this muscle preserves its ability to contract in a hypotonic (0·112 M) solution of sucrose for 24 hr; this is accompanied by an action potential. In this period the muscle loses almost all its sodium but preserves more than 30 per cent of its potassium.

The permeability of the smooth stomach muscle of the frog *Rana pipiens* for non-electrolytes and electrolytes was studied in detail by Bozler and his colleagues (Bozler, 1959; Bozler, Calvin and Watson, 1958; Bozler and Lavine, 1958). It was found (Bozler, Calvin and Watson, 1958) that the water content of this muscle is 80·4 per cent and the inulin space 27·8 per cent.

The concentration of inorganic ions in this muscle after the establishment of equilibrium in Ringer's solution was (in mequiv/kg of intracellular water): chloride—14, sodium—66, potassium—129. In distilled water the muscles double in volume in 1 hr; in these conditions they lose almost all their sodium and chloride and up to 80 per cent of their potassium. Muscles placed in a solution of 2 mM calcium chloride swell less and lose less potassium and sodium. They can preserve up to 80 per cent of their potassium, though the permeability of the muscles is strongly increased for electrolytes and even for inulin.

Muscles previously loaded with radioactive potassium lose it into a non-radioactive Ringer's solution in two phases: 4 per cent of the intracellular radioactive potassium is released very quickly from the muscles, the remainder being lost slowly by the muscle fibres.

Later (Bozler and Lavine, 1958) it was found that in 0.22 M sucrose solution at 25° the stomach muscles release their chloride completely in 20 min and their sodium in 1 hr, whereas in these conditions 15–20 per cent of the potassium is released in 15–20 min and its concentration in the muscle remains unchanged.

On the basis of the results obtained Bozler and his colleagues came to the conclusion that the regulation of the permeability and osmotic processes of these muscles is achieved not only by cell membranes (or not so much by

TABLE 73. THE MINERAL COMPOSITION OF THE PLASMA, SMOOTH AND STRIATED FROG MUSCLES  
(in mg per cent, dry residue and water in per cent)

Subject	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	P	Cl <sup>-</sup>	Dry residue	Water	Author
Striated muscles	310	55	16	24	186	44	18.3	81.7	Katz (1896)
Smooth muscles	320	75	4	13	135	120	18.0	82.0	Meigs and Ryan (1912)
Blood plasma	9.8	238.7	8.0	7.3	9.6	262.7	4.0	96.0	Fenn (1936)

them) but by the cell protoplasm as a whole. These authors supposed that the water in the protoplasm structures is not equally available for the solution of different substances, and that the accumulation of potassium is achieved by the muscles owing to specific adsorption by the cell colloids, as occurs in ion-exchange resins.

According to the experiments of Jendrassik (1924) smooth muscle fibres of warm-blooded animals are also permeable for salts.

Born and Bülbbring (1956), using radioactive potassium, studied the exchange in various experimental conditions of potassium from strips of the large intestine of guinea pigs for potassium from the surrounding salt solution. They found that the half period of mixing of potassium of pieces of intestine with labelled potassium from the medium  $t_{\frac{1}{2}} = 55$  min. Contraction of the intestinal muscles is accompanied by increased rate of release of labelled potassium from the muscles and decreased access of it to the tissue. The same effect is produced by histamine and acetylcholine. Adrenalin has no noticeable effect on this process.

In similar experiments on the same subject Hurwitz (1959) showed that pilocarpine in very small concentrations also increases the rate of release of  $^{42}\text{K}$  into a non-radioactive solution from the muscles and depresses its rate of entry into the muscles. Cocaine reduces the effect of pilocarpine.

Goodford and Hermansen (1960) studied the dynamics of sodium and potassium exchange for strips of muscle from isolated guinea pig small

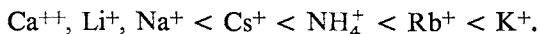
intestine. They found that after incubation of the pieces in Krebs' solution the concentration in the tissue was: sodium 100 and potassium 68 mm/kg of wet weight; there was about 80 per cent water; the inulin space was 33 per cent. The values obtained did not change in the course of 6 hr incubation. The specific radioactivity of the sodium of the pieces of intestine on incubation in a radioactive Krebs' solution grows very quickly ( $t_{\frac{1}{2}} = 1$  min). The process of exchange of tissue potassium for radioactive potassium from the medium consists of one fast and one slow component ( $t_{\frac{1}{2}}$  is 50 min and 8 hr respectively).

Some interesting results are reported in a paper by Dawkins and Bohr (1960). They found that pieces of isolated rat aorta on incubation in Krebs' solution lose a large amount of potassium and absorb a large amount of sodium in the first 2 min. However, after this period the reverse process is observed: the sodium is released from the tissue, while the potassium passes into it, so that after half an hour the concentration of potassium and sodium in the pieces of aorta approximates to the original level. The same is seen on incubation of pieces of aorta in rat blood plasma. The effect does not occur if the rats are perfused with Krebs' solution.

#### *The permeability of nerve fibres for ions.*

Individual nerve fibres behave in respect of their permeability for salts just like muscle fibres. From the point of view of the membrane theory they are impermeable for anions but permeable for certain cations.

Thus Netter (1927), studying the effect of various salts on the magnitude of the resting current of frog nerve, came to the conclusion that nerve fibres are impermeable for anions and permeable to varying degrees for cations. From the rate of penetration of cations, i.e., in the present case from the degree of depression of the resting current, the author obtained a series similar to that obtained by Höber (1905) in his muscle experiments, namely:



Calcium, lithium and sodium ions are as it were completely incapable of penetrating nerve fibres, while of the remaining cations the one to penetrate at the greatest rate is potassium. The inequality of the concentrations of the individual ions in the nerve and outside would be a further indication of the impermeability of the membrane of these fibres for salts.

The electrolyte composition of frog sciatic nerve was investigated in detail by Fenn, Cobb, Hegnauer and Marsh (1934-5). Their results are shown in Table 74.

The amount of chloride in the nerve is about half that in the plasma. Assuming that the chloride is found only in the intercellular spaces, the authors determine the magnitude of the latter as 50 per cent of the nerve weight. Hence it follows that of the total amount of ions in the extracellular spaces there is so much that there should be half the amount in the plasma, the

remainder being inside the nerve fibres. On this basis Fenn and his colleagues built up their table.

However, if attention be paid to certain details of this table, substantial divergences will easily be found between the experimentally obtained data and the theory which the authors uphold. Thus, it is completely unintelligible that the nerve contains only 0.08 mM  $\text{HCO}_3^-$  per 100 g nerve, whereas it should contain a minimum of 1.27 mM. Still further discrepancies arise if account is taken of the fact that the chloride ion concentration in the nerve fibre reaches 30 per cent of its external concentration.

TABLE 74. THE REDISTRIBUTION OF MINERAL SUBSTANCES BETWEEN NERVE AND PLASMA OF FROGS (in mM per 100 g of nerve or 100 g of plasma) (From Fenn, Cobb, Hagnauer and Marsh 1934/5)

Mineral substances	in blood plasma	in nerve	in intercellular (chloride) space	in nerve fibre
Sodium	10.38	6.20	5.19	1.01
Potassium	0.25	4.80	0.13	4.67
Ammonium	—	0.19	—	0.19
Calcium	0.20	0.36	0.10	0.26
Magnesium	0.30	0.80	0.15	0.65
Chloride	7.43	3.70	3.70	—
Phosphate	0.31	1.00	0.15	0.85
Lactate	0.80	0.80	0.40	0.40
Bicarbonate	2.54	0.08	1.27	—
Sulphate	—	0.35	—	0.35
Water	96.00	75.00	48.00	27.00
mm/100 cm <sup>3</sup> $\text{H}_2\text{O}$	23.2	25.7	23.2	31.0
Cations (in equiv)	11.63	13.51	5.81	7.69
Anions (in equiv)	11.39	7.93	5.69	2.45

The authors established that chloride and sodium are released completely from the nerve into an isotonic glucose solution in a short time, while in the same time the nerve loses only an insignificant amount of potassium: it is incapable of releasing it completely as long as it remains alive. These experiments show convincingly that both potassium and sodium, which is found inside the nerve too, are able to diffuse inside the nerve fibre and out of it.

Fenn and his colleagues paid much attention to the permeability of nerve fibres for potassium. On immersing isolated nerve for 5 hr in Ringer's solution containing definite amounts of potassium (from 0 to 12 mequiv per cent), they discovered absorption of potassium in solutions containing more than 1.6 mequiv per cent and release into the solution at lower concen-

trations. The absorption of potassium by the nerve is directly dependent on the potassium concentration in the medium. Similar phenomena were observed by the authors in experiments with crab and lobster nerve. They also established that absorption or release of potassium by the nerve is not dependent on the hydrogen ion concentration in the medium. On immersing nerve in Ringer's solution at pH 6·3, these authors observed strong absorption by the nerve of sodium and chloride and insignificant release of potassium.

Later investigations by many writers showed that sodium and chloride penetrate freely not only into the tissue space of nerve, but also inside the nerve fibres, in which they are constantly present, though in lower concentrations than in the liquid surrounding the nerves. Thus, for example, Shanes (1954a) showed that the nerve fibres of the sciatic nerve of the toad *Bufo marinus* are permeable for sodium and chloride ions; the permeability of the nerve fibres of this same animal for potassium and sodium was established by Shaw and Simon (1955). Sodium and potassium ions, as was shown by MacLennan and Harris (1954), can penetrate the fibres of rabbit sciatic nerve; these scientists found that at 0° the sodium concentration in the nerve is increased: at 18° all the sodium of the nerve is exchanged for radioactive sodium ( $^{24}\text{Na}$ ) of Ringer's solution, whereas the nerve potassium is only 36 per cent exchanged. However, in a phosphate (potassium) Ringer's solution all the nerve potassium is exchanged in 1 hr. The authors suppose that part of the potassium in the nerve is somehow bound.

Detailed studies of the permeability of the fibres of myelinated nerves for potassium, sodium, chloride and other ions have been made by a series of workers (Euler *et al.*, 1946; Graude and Richter, 1949; Harreveld, 1950, 1951; Shanes, 1952, 1953, 1954a, b; Shanes and Berman, 1953, 1955a, b, 1956; Krnjević, 1954, 1955; Danty and Krnjević, 1955; Mullins and Grenell, 1952; Mullins, 1954; Niedetzky, 1959 and others). Euler, Euler and Hevesy (1946) found that radioactive potassium, sodium, bromide and orthophosphate ions, when introduced into cat blood, penetrate the nerve fibres (like the cells of other tissues), stimulation of the nerve leading to enhanced permeability of the fibres for these ions. However, Niedetzky (1959) connects this effect with damage of the nerve on direct stimulation by electric current. Mullins (1954), studying the inclusion of the phosphorus of labelled orthophosphate into the sciatic nerve of the frog *Rana pipiens* in different experimental conditions, also found no enhancement of the absorption of radioactive phosphorus upon stimulation of the nerve.

Particularly convincing are the experiments conducted on individual giant nerve fibres of certain cephalopod molluscs. The diameter of these fibres is as large as 0·3–1·0 mm thus allowing chemical analysis of the actual axoplasm after extraction from the nerve fibre by pipette. In Table 75, which was compiled by Hodgkin (1958), the results are given of the analysis of the axoplasm and plasma of squid blood.

It can be seen from Table 75 that in the axoplasm the potassium concentration is 20 times greater than in the blood plasma. The axoplasm of this nerve contains a considerable amount of sodium and chloride; however, there is only about one eighth of the amount of the former in the axoplasm and one fourteenth of the latter as compared with the blood plasma. About the same ratio of ion concentrations holds between the axoplasm and sea water.

TABLE 75. THE APPROXIMATE CONCENTRATION OF MINERAL IONS AND OTHER SUBSTANCES IN THE AXOPLASMA OF FRESHLY ISOLATED GIANT AXONS AND IN THE BLOOD PLASMA OF THE SQUID (in mm/kg of water; water in g/kg) (from Hodgkin, 1958)

Substance	Concentration		
	in axoplasm	in blood	in sea water <sup>(1)</sup>
H <sub>2</sub> O	865 <sup>(2)</sup>	870 <sup>(3)</sup>	966 <sup>(6)</sup>
K <sup>+</sup>	400 <sup>(4)</sup>	20 <sup>(5)</sup>	10 <sup>(6)</sup>
Na <sup>+</sup>	50 <sup>(4)</sup>	440 <sup>(7)</sup>	460 <sup>(6)</sup>
Cl <sup>-</sup>	40 <sup>(8)</sup>	560 <sup>(7)</sup>	540 <sup>(6)</sup>
Ca <sup>++</sup>	0.4 <sup>(9)</sup>	10 <sup>(7)</sup>	10 <sup>(6)</sup>
Mg <sup>++</sup>	10 <sup>(10)</sup>	54 <sup>(7)</sup>	53 <sup>(6)</sup>
Isethionate	270 <sup>(2)</sup>	—	—
Aspartic acid	75 <sup>(2)</sup>	—	—
Glutamic acid	12 <sup>(2)</sup>	—	—
Succinate + fumarate	17 <sup>(2)</sup>	—	—
Orthophosphate	2.5-9 <sup>(11)</sup>	—	—
Adenosine triphosphate	0.7-1.7 <sup>(11)</sup>	—	—
Phosphagen (Creatine Phosphate)	1.8-5.7 <sup>(11)</sup>	—	—

Notes: <sup>(1)</sup> salinity = 3.45 per cent; <sup>(2)</sup> Koechlin, 1955; <sup>(3)</sup> Robertson, 1949; <sup>(4)</sup> Steinbach and Spiegelman, 1943; Keynes and Lewis, 1951a; <sup>(5)</sup> Robertson, 1949; <sup>(6)</sup> Webb 1939, 1940; <sup>(7)</sup> Robertson, 1949; <sup>(8)</sup> Steinbach, 1941; <sup>(9)</sup> Keynes and Lewis, 1956; <sup>(10)</sup> Koechlin, 1955; <sup>(11)</sup> Caldwell, 1956.

Keynes and Lewis (1951a) found in the axoplasm of resting squid nerve: 323 mm of potassium, 46 mm of sodium and about 72 mm of chloride per kg of axoplasm. Somewhat smaller values for potassium, sodium and chloride are reported by Webb and Young (1940).

Steinbach (1941) and Steinbach and Spiegelman (1943) found in the axoplasm of squid nerve 369 mm of potassium, 44 mm of sodium and 36 mm of chloride per kg of axoplasm. If the nerve is placed in sea water, the amount of potassium in it falls, while the amounts of sodium and chloride rise. Thus, after 1 hr in sea water there was found in the nerve: potassium — 321 mm, sodium — 101 mm/kg of axoplasm. In these conditions the chloride concentration in the axoplasm rises very quickly: in 30 min the amount in the axoplasm reaches 75 mm, remaining constant thereafter for a long time,

so that equality of chloride concentrations in the axoplasm and medium is not achieved (there is 540 mm of chloride in the medium). In an isotonic sugar solution and in chloride-free salts solutions the nerve fibre for a short time loses all its chloride, but if the nerve is then replaced in sea water, this anion appears in its former concentration in the axoplasm.

On incubation of isolated nerves or nerve fibres in Ringer's solution and in sea water in various conditions there is always observed to be absorption by the nerve fibres of sodium and chloride ions and release of potassium ions by them into the medium (Arnett and Wilde, 1941; Fenn and Gerschman, 1950; Harreveld, 1951; Harreveld and Russell, 1954; Hodgkin and Huxley, 1953; Abbott, Hill and Howarth, 1958 and others). Thus Hodgkin and Huxley (1953) established that isolated giant nerve fibre of the cuttlefish *Sepia officinalis*, when immersed in sea water, loses its potassium (in the first hours of the experiment) at a rate of 55 pm/cm<sup>2</sup> sec. Keynes and Lewis (1951a) showed that the isolated axon of the cuttlefish at low temperature in the resting state absorbs sodium from sea water at a rate of 8.8 mm/kg of axon per hour and loses potassium at approximately the same rate. Keynes and Lewis (1951b) found in crab nerve 257–268 mm/kg of potassium and 152 mm/kg of sodium. They supposed that the sodium is partially inside the cells and its concentration there is the same as in squid axoplasm (40 mm/l). Hence the authors drew the conclusion that the tissue space of crab nerve is 25 per cent of the whole weight of the nerve and, consequently, there will be 342 mm/kg of potassium in the axoplasm. Using radioactive potassium they found that isolated bundles of crab nerve on immersion in Ringer's solution lose <sup>42</sup>K at a rate of 22 pm and at the same time absorb this ion from the medium at a rate of 19 pm/sec/cm<sup>2</sup> of fibre surface. On immersion of the nerve in potassium-free Ringer's solution the rate of flux was significantly reduced.

Keynes (1951b) studied the rate of flux of radioactive potassium and sodium ions into and out of isolated giant nerve fibres of cuttlefish. It was found that potassium passes into the fibres at a rate of 17 and out at a rate of 58 pm per sec per cm<sup>2</sup> of fibre surface, while the corresponding figures for sodium are 61 and 31 pm.

Rothenberg (1948a, b, 1950) and Hodgkin and Keynes (1955a, b, 1956) studied the rate of exchange of potassium and sodium of the giant fibres of the squid and cuttlefish for the same ions from artificial sea water in various experimental conditions (high and low temperature, action of enzymatic poisons, etc., see below). Hodgkin and Keynes (1955a) found that the potassium ion flux into the cuttlefish axon is 13 and that out 29 pm/cm<sup>2</sup> sec (external potassium concentration 10.4 mm, internal 300 mm/kg of axoplasm, temperature 17°). After excitation of the axons by electric current and the passage along the fibre of 10,000–40,000 impulses, the sodium ion flux into the fibre is 32 and out 39 pm/cm<sup>2</sup> sec, the corresponding figures for potassium being 21 and 28 pm/cm<sup>2</sup> sec. The inter-

nal potassium concentration at the end of the experiment was 267, the sodium concentration 77 mM/kg of axoplasm; the external sodium concentration was 486 mM, the temperature 18°.

Shanes and Berman (1955c) give the following data for the ion flux into the giant fibre of squid: potassium 47, sodium 63, chloride 14, and sodium out of the fibre into the medium  $33 \text{ pm/cm}^2 \text{ sec}$ . The calcium ion flux into this fibre when the magnesium ion content of the medium is 55 mM is about  $0.1 \text{ pm/cm}^2 \text{ sec}$ . The rate of penetration of calcium ions into the fibre depends on the concentration of magnesium and potassium ions in the medium (Hodgkin and Keynes, 1957). At a concentration of labelled calcium ions ( $^{45}\text{Ca}$ ) in the medium of 10.7 mM the calcium flux into the giant squid fibre in a period of from 35 to 170 min was  $0.05\text{--}0.4 \text{ pm/cm}^2 \text{ sec}$  (Flückiger and Keynes, 1955). This value of calcium ion flux is considerably lower than that given by Rothenberg (1950). If the calcium ion concentration in the medium is raised, and also if the nerve is excited, the calcium flux into the fibres of the squid and crab nerve grows considerably (Keynes and Lewis, 1956).

Tobias (1948) established the permeability of cockroach nerve fibres for potassium, sodium and chloride ions.

On the basis of the evidence cited above one may with confidence assert that nerve fibres, just like muscle fibres, are permeable for both cations and anions.

*The permeability of epithelial, nerve and other cells of animal organisms for ions.* The permeability for inorganic substances of epithelial, nerve, junction tissue and other cells of multicellular organisms has been studied much less thoroughly than the permeability of the formed elements of the blood and of muscle and nerve fibres. This is explained chiefly by methodical difficulties connected with the morphological complexity of the structure of the organs of which these cells form part. Nonetheless, even the scanty published material available on the problem leaves no room for doubt about the fact that there is no difference in principle between the permeability of the formed elements of the blood and of muscle and nerve fibres and that of any other cells in multicellular organisms. All the inorganic ions that are found in epithelial, nerve, junction tissue, sexual and other cells are continuously exchanged for the same ions from the tissue fluid bathing these cells, this exchange continuing both in the resting and in the active state of the cells. This has been demonstrated completely satisfactorily by the use of labelled atoms and other methods.

Thus Hevesy and Euler (1942) introduced radioactive phosphate subcutaneously into rats with a sarcoma; after 2 hr they found in the various organs of the animal amounts of  $^{32}\text{P}$  as shown in Table 76.

These workers found that 1 kg of rat plasma contained about 50 mg of free phosphorus. Hence it follows that in 2 hr 50 mg of phosphorus passed into 1 kg of brain, almost twice as much into the same amount of muscle tissue, 26 times as much into the liver tissue, 12 times as much into the

spleen tissue, 24 times as much into the testicles and 15 times as much into the sarcoma cells as passed into the brain cells. This difference in the amount of phosphorus absorbed by the various tissues indicates varying levels of phosphorus exchange (principally exchange of organic phosphorus) in the cells of these organs. Growing cells (sarcoma cells) and cells with a high level of secretory activity (liver cells) absorb, as can be seen from the table, the largest amounts of phosphorus.

TABLE 76. THE AMOUNT OF RADIOACTIVE PHOSPHORUS IN VARIOUS RAT ORGANS  
2 HR AFTER THE SUBCUTANEOUS INTRODUCTION OF  $\text{Na}_2\text{H}^{32}\text{PO}_4$   
(from Hevesy and Euler, 1942)

Organ	Ratio of the $^{32}\text{P}$ content of the tissue to the $^{32}\text{P}$ content of the plasma
Liver	26
Spleen	12
Muscles	1.9
Testes	2.4
Brain	1.0
Sarcoma	15

Similar results were obtained by Hevesy and Hahn (1940) upon intravenous introduction into rabbits of labelled phosphorus: they compared the specific activity after a fixed time interval of the inorganic phosphate extracted from the various tissues and organs with that of the inorganic phosphate in the plasma. The results obtained by Grodzenskii and Il'yina (1940, 1941) point in the same direction. One may also mention the work of Ginsburg and Wilde (1954) and of Odeblad and Ziliotto (1955), who studied the distribution of radioactive phosphorus in various organs of rats, rabbits, guinea pigs and mice.

The fact that the passage of many substances, including inorganic ions, from the blood into the cells of the central nervous system is achieved more slowly than that into the cells of other organs and tissues has been known for a long time. Dawson (1952), using radioactive phosphorus, showed that the slow passage of phosphorus into the brain is caused by the slow passage of this ion out of the chorioidal plexus. On passing into the central nervous system, the phosphorus quickly penetrates the nerve cells and is exchanged with the phosphorus groups of the phosphocreatine and adenosinetriphosphate; it is incorporated more slowly into the phospholipids and nucleoproteins. Streicher (1956) and Verzhbinskaya (1957) produced a large amount of experimental material which shows that the passage of orthophosphate from the blood channel into the brain cells is limited by the rate of incorporation

of phosphorus into the organic phosphorus compounds of the brain cells, and not by any diffusion barrier separating the brain from the blood.

Smirnov and Chetverikov (1953a, b), using  $^{32}\text{P}$  to investigate the exchange of phosphorus in the central nervous system of rabbits, established that in order of decreasing rate of phosphorus incorporation the parts of the central nervous system can be arranged in the following series: cerebral cortex, cerebellum, middle brain, medulla oblongata, spinal cord.

The passage of labelled orthophosphate from the blood into the brain was studied in the case of rats in normal conditions and in deep hypothermy ( $18\text{--}20^\circ$ ) by Vladimirov *et al.* (1956, 1959).

Brain sections and eye retinae of guinea pigs (Krebs *et al.*, 1951), brain sections of guinea pigs and hamsters (Garoutte and Aird, 1955, 1956), and guinea pig brains (Manery and Husdan, 1956), when incubated in salt solution, lose a considerable amount of potassium and absorb sodium and chloride ions. In these experiments rapid exchange is observed of the cell potassium for labelled potassium from the medium.

Harris and McLennan (1953) and McLennan (1953a, b) studied the dynamics of potassium and sodium exchange in the case of isolated sympathetic ganglia (superior cervical ganglia) of rats, rabbits, cats and dogs. On the average, these ganglia in rats and rabbits contain  $89 \pm 23$  mequiv/kg of potassium,  $81 \pm 14$  mequiv/kg of sodium and  $92 \pm 29$  mequiv/kg of chloride. Assuming that the sodium and chloride are located extracellularly, they calculated that the extracellular space of the ganglia is 50–60 per cent and that the intracellular concentration of potassium will be 250–350 mequiv/kg of cell water. Using radioactive sodium they found that its rate of diffusion into the ganglia is 50 times slower than free diffusion in water. They found further that only about 45–50 per cent of the intracellular potassium is exchanged for  $^{42}\text{K}$  from the medium, of which 10 per cent is exchanged rapidly and 35–40 per cent slowly. The mean time constant of potassium exchange for ganglia is for rats 0.68, for rabbits 0.23, for cats 0.17 and for dogs  $0.13 \text{ hr}^{-1}$ . In an isotonic potassium phosphate solution all the potassium of the ganglia is very quickly exchanged for labelled potassium from the salt solution.

Many studies have been made of the mineral composition and its metabolic regulating mechanism for kidney sections of different animals (Conway, Fitzgerald and MacDongald, 1946; Robinson, 1950, 1952; Boatman *et al.*, 1960; Krebs, Eggleston and Terner, 1951; Mudge, 1951a, b, 1952; Aebi, 1952a, 1953; Deyrup, 1953a, b; Whittam and Davies, 1953, 1954; Davies, 1954; Leaf *et al.*, 1954, 1956; Whittam, 1956, 1960; Cort and Kleinzeller, 1956, 1957, 1958; Kleinzeller and Cort, 1957a, b, 1960; Mounib and Evans, 1960; Kleinzeller, 1960, 1961; Foulkes and Miller, 1961). In these papers it was shown in general that, as in experiments with other organs and tissues, on the incubation of sections in salt solution the breakdown of the normal flow of cellular metabolism (lowering of temperature, exclusion from the

medium of the substrates of enzymatic reactions, effect of metabolic inhibitors) leads to release by the cells of potassium and their enrichment in sodium, chloride and water slices. This process is reversible. The results quoted show convincingly that a definite mineral composition is preserved in the cells of the slices owing to the energy of metabolic processes. In Tables 77 and 78, compiled by Harris (1960) from the results of a number of

TABLE 77. THE MINERAL COMPOSITION OF THE KIDNEY OF CERTAIN VERTEBRATES  
(in mm/kg of fresh weight, water in g/kg) (from Harris, 1960)

Type of animal	Potassium	Sodium	Chloride	Water	Author
Dog whole kidney <sup>(1)</sup>	58.3 ± 4.0	82.6 ± 5.8	67.7 ± 5.3	802 ± 5.6	Eichelberger and Bibler (1940)
cortex	65.4	75.1	64.1	789	
medulla	49.4	124.9	115.0	824	
Rat	71.8	62.5	—	770	Aebi (1953)
Rabbit	69.3 ± 6.88	68.5 ± 6.5	74.8	771 ± 1.0	Mudge (1951a)
Rabbit	76.8	73.3	—	772	Aebi (1953)
Guinea pig	76.4	63.4	—	777	Whittam and Davies (1953)
Guinea pig	81.1	61.8	—	782	Aebi (1953)
Frog	60.0 ± 0.6	41.2 ± 0.6	30.1 ± 0.8	816	Conway <i>et al.</i> (1946)

<sup>(1)</sup> Calcium = 2.16 ± 0.53; Mg = 5.2 ± 0.5; ± standard error.

TABLE 78. THE CHANGES IN THE POTASSIUM AND SODIUM CONCENTRATIONS IN SLICES OF KIDNEY ON INCUBATION (± s.e.) (from Harris, 1960)

	Rat <sup>(1)</sup>	Rabbit <sup>(1)</sup>	Guinea pig <sup>(1)</sup>	Guinea pig <sup>(2)</sup>
<i>Fresh slices</i>				
Dry weight, %	23	22.8	21.8	22.3 ± 0.2
K <sup>+</sup> , mm/kg of tissue	71.8	76.8	80.1	76.4 ± 0.9
Na <sup>+</sup> , mm/kg of tissue	62.5	73.3	61.8	63.4 ± 1.9
K <sup>+</sup> — Na <sup>+</sup> , mm/kg of water of slices	174.5 ± 3.5	194.3 ± 4.1	181.5 ± 3.8	179.7 ± 2.6
<i>After incubation for 1 hr in saline solution</i>				
Dry weight, %	20.9	25.1	24.2	20.3 ± 0.3
K <sup>+</sup> , mm/kg of tissue	71.1	97.9	75.6	75.3 ± 1.6
Na <sup>+</sup> , mm/kg of tissue	133.7	110.8	98.1	90.2 ± 1.8
K <sup>+</sup> — Na <sup>+</sup> , mm/kg of water of slices	259.5 ± 3.5	279.0 ± 10.8	229.2 ± 9.5	207.5 ± 3.0

<sup>(1)</sup> From Aebi (1953).

<sup>(2)</sup> From Whittam and Davies (1953).

workers, we show the mineral composition of the kidney slices and its change on incubation of the slices in salt solution.

The inulin space of kidney slices of rats and guinea pigs at 37° is 21 per cent and at 0° 16 per cent (Conway and Geoghegan, 1950), but according to the data of other authors it is the same at high and low temperatures — 26 per cent (Robinson, 1950).

TABLE 79. THE KINETICS OF THE ENTRY OF CHLORIDE-36 IONS INTO SLICES OF GUINEA PIG KIDNEY CORTEX DURING INCUBATION IN SALINE SOLUTION AT 37°  
(from Whittam, 1956)

Exchange index	Experimental conditions: in an atmosphere		
	of oxygen	of $O_2 + 0.2\text{ mm}$ of DNP	oxygen-free
$\text{Cl}^-$ concentration in slices (mequiv/kg of tissue)	55.7	77.7	73.5
Volume of space of quickly exchanging chloride fraction (ml/g)	0.27	0.51	0.27
Intracellular concentration of chloride ions (slowly exchanging fraction of chloride-mm/kg of tissue $[\text{Cl}^-]_i$ )	31	31	55
Constant of rate of exit of slowly exchanging chloride, $K_0$ ( $\text{min}^{-1}$ )	0.47	0.074	0.1
$t_{\frac{1}{2}}$ of exchange of slow chloride fraction (min)	1.5	9.4	3.3
Rate and exchange of chloride ions of slow fraction ( $m_0 = K_0 [\text{Cl}^-]_i$ ; mequiv/g·min)	14	2.3	11

Whittam (1956) studied the process of chloride ion exchange between slices of guinea pig kidney and a saline medium. The author established that this process is made up of two exponential components, there being a quickly exchanging and a slowly exchanging fraction of the chloride ions. The quickly exchanging fraction (half time — 15 sec) corresponds to the chloride concentration of the intercellular spaces, as determined by inulin (27 per cent). Similar data had earlier been obtained for sodium exchange (Whittam and Davies, 1954). The volume and rate of exchange of the slow and quick fractions of the chloride ions in kidney slices changes depending on the experimental conditions. The figures in Table 79 present a clear picture of this.

In a series of papers it was shown that the exchange of cell sodium and potassium in kidney slices for the corresponding radioactive isotopes of sodium and potassium from the medium is not governed by any simple exponential law, which indicates the presence of some intracellular barriers for the free diffusion of sodium and potassium into the cell and from

the cell outward. (Mudge, 1952, 1953; Whittam and Davies, 1954). Mudge (1952) postulates the existence within the cell of potassium chemically bound with the proteins.

According to Cort and Kleinzeller (1957), the concentration of  $\text{Na}^+$  in the cells of kidney slices is regulated actively, while that of  $\text{Li}^+$ ,  $\text{K}^+$  and  $\text{Rb}^+$  is regulated passively.

The cells of liver slices on incubation in salt solution behave in respect of ion exchange and change in their concentrations depending on the conditions of incubation in just the same way as cells of brain, kidney and other slices (Flink, Hastings and Lowry, 1950; Aebi, 1953; Geyer, Sholtz and Bowie, 1955; Bass and Saltman, 1959; McLean, 1960; Broome and Opie, 1960; Burck, 1961, etc.). In Table 80 we reproduce data from the paper by

TABLE 80. THE AMOUNT OF SODIUM, POTASSIUM AND WATER IN SLICES OF THE LIVER OF ANIMALS BEFORE AND AFTER 1 HR INCUBATION IN SALINE SOLUTION AT BODY TEMPERATURE (from Aebi, 1953)

Animal	Before incubation				After incubation	
	% water	$\text{K}^+$	$\text{Na}^+$	Total	% water	total bases ( $\text{K}^+ + \text{Na}^+$ ) mm/kg of tissue
		m/kg of fresh weight of tissue				
Rat	69.3	95.2	27.2	176.5	74.8	216.2
Guinea pig	71.2	89.6	32.5	171.5	75.6	225.5
Rabbit	71.2	69.2	43.5	158.0	74.1	208.3

Aebi (1953) on the bases and water content of liver slices from a number of animals before and after incubation in a saline medium. The general raising of the concentration of bases in the slices after incubation occurred because of strong absorption by the slices of sodium ions from the incubational saline solution.

Grodzenskii and Kiseleva (1949) established that there is very rapid penetration of labelled phosphate into rat liver cells, and Guberniyev and Il'yina (1950) found the same for the cells of dog digestive organs.

Morton and Schwartz (1953) showed that the cells of live slices of thyroid gland, liver and kidney, when incubated in the presence of  $^{32}\text{P}$ , are permeable for phosphate. The same was observed by Hokin and Hokin (1953) in experiments with live slices of the pancreas, liver and brain of pigeons and the liver, ventricles of the heart, kidney cortex and cerebral cortex of the guinea pig.

The permeability of the cells of different organs for mineral ions may also be determined by the change in the ratios of the concentrations of the various ions in the organ and the plasma. Manery and Bale (1941) and Manery and Haege (1941) used both chemical and radioisotope techniques to determine

the "extracellular aqueous spaces" of various rat and rabbit tissues and organs. The radioactive substances were injected into the rabbit in the ear vein and into the rat peritoneally.

Table 81 gives the magnitudes of these spaces in rat tissues as determined chemically from the chloride distribution and from the distribution of radioactive phosphorus introduced intraperitoneally in the form of  $\text{Na}_2\text{HPO}_4$ , and the change with time of the " $^{32}\text{P}$  space".

TABLE 81. THE CHLORIDE AND RADIOACTIVE PHOSPHORUS "SPACES"  
IN RAT TISSUES AND ORGANS (from Manery and Bale, 1941)

Tissues and organs	Time after introduction of $^{32}\text{P}$						
	7 min		14 min		20 min		2 hr
	Amount of $(\text{H}_2\text{O})_E$ in g/100 g of tissue found from chloride or from radioactive phosphorus						
	$\text{Cl}^-$	$^{32}\text{P}$	$^{32}\text{P}$	$\text{Cl}^-$	$^{32}\text{P}$	$\text{Cl}^-$	$^{32}\text{P}$
Skin	51.4	33	29	45.2	38	51.5	98
Kidneys	41.8	—	104	48.5	242	45.3	788
Liver	23.5	—	90	30.3	193	26.1	1007
Testes	51.4	58	56	55.0	30	52.9	50
Gastrocnemius muscle	12.2	12	10	14.6	12	14.0	73
Stomach	58.8	—	89	53.3	124	51.5	388
Heart	24.7	—	48	28.6	65	23.6	265

In the same papers Manery and his colleagues give the "extracellular aqueous spaces" ( $\text{H}_2\text{O})_E$  in rabbit and rat organs for chloride, sodium, radioactive chloride ( $^{38}\text{Cl}$ ) and radioactive sodium ( $^{24}\text{Na}$ ). The relevant data for rabbits are set out in Table 82. Similar results were obtained for rat organs.

These tables show that for some organs the "chloride space" is greater than the "sodium space", while for others the reverse is the case. The values of  $(\text{H}_2\text{O})_E$  obtained by using radioactive sodium and chloride indicate that only a few minutes in all are required for the chloride and sodium "spaces" to become exchanged with the isotopes in all the organs except the central nervous system. This means that chloride and sodium, which are present both inside and outside the cells, are very rapidly exchanged for the same ions from the plasma, which points to a high degree of permeability for the cells of the various organs for these ions.

Unfertilised eggs of echinoidea and certain other sea animals were shown by Brooks (1939a, 1940, 1943a, b) to be freely permeable for phosphate, chloride, sodium, potassium and rubidium. Equilibrium between the radioactive sodium added to the sea water and the radioactive sodium penetrating the egg cells is reached in 2–15 min. Similar results were obtained by Abelson and Duryee (1949) in experiments on frog eggs using  $^{24}\text{Na}$ .

Lillie (1916) established that the permeability of sea urchin eggs for water is strongly increased on fertilisation or artificial activation. Then Needham and Needham (1940) found increased permeability of cells for phosphate in the various stages of embryonic development of a number of invertebrates.

TABLE 82. THE SIZES OF THE "SPACES" OF CHLORIDE, RADIOACTIVE CHLORIDE, SODIUM AND RADIOACTIVE SODIUM IN RABBIT ORGANS

(from Manery and Bale, 1941 and Manery and Haege, 1941)

Tissues and organs	Time after introduction of isotopes							
	1 hr 8 min		13 min		11 min		48 min	
	Isotopes used to determine $(H_2O)_E$ (g/100 g of tissue)							
	Cl <sup>-</sup>	Na <sup>+</sup>	<sup>24</sup> Na <sup>+</sup>	<sup>24</sup> Na <sup>+</sup>	Cl <sup>-</sup>	<sup>38</sup> Cl <sup>-</sup>	Cl <sup>-</sup>	<sup>38</sup> Cl <sup>-</sup>
Skin	62.5	—	—	42	60.6	56	62.6	—
Tendon	74.6	52.1	53	39	51.7	56	—	66
Kidneys	51.3	45.8	42	33	38.8	41	45.4	59
Testes	48.5	28.4	28	18	—	—	—	—
Ear cartilage	55.6	96.6	104	71	50.2	37	48.5	58
Spleen	43.0	30.1	27	23	—	—	—	—
Liver	22.3	22.6	22	17	19.3	18	21.4	25
Mucous pylorus	67.1	33.1	36	33	—	33	58.9	38
Pylorus muscles	43.3	25.7	29	23	40.4	30	43.8	—
Thin intestine	37.5	33.2	32	—	—	—	—	—
Sciatic nerve	45.7	54.2	28	—	—	—	—	—
Cerebrum	36.8	35.9	11	4.3	31.8	4.7	33.0	11
Bladder	57.8	43.9	40	49	—	—	—	—
Heart	29.5	29.7	30	28	29.6	31	—	—
Stomach muscle	21.8	17.7	19	11	15.8	14	—	—
Gastrocnemius muscle	12.1	10.0	10	7.7	11.6	8.3	11.4	0.4

In recent years this problem has been studied in detail by techniques involving labelling of substances with radioactive or heavy isotopes. Thus, in experiments with the eggs of various kinds of sea urchins, stars, worms, fish, amphibians and birds, Brooks (1939a, 1943a, b), Abelson (1947, 1948), Lindberg (1948, 1950), Brooks and Chambers (1948, 1954), Chambers and White (1949, 1954), Chambers, Whiteley, Chambers and Brooks (1948), Whiteley (1949), Villee, Lowens *et al.* (1949), Lansing and Rosenthal (1952), Chambers and Mende (1953a, b) and Vincent (1954) established that orthophosphate labelled with <sup>32</sup>P passed slowly into unfertilised egg cells; but several minutes after fertilisation the rate of penetration of this anion grows tens and hundreds of times.

According to Brooks and Chambers (1954) and Chambers and White (1954) the eggs of *Strongylocentrotus purpuratus* and *S. franciscanus* absorb radioactive orthophosphate from the medium up to fertilisation at a rate of

0.0015–0.0041, and after fertilisation at a rate of 0.11–0.54  $\mu\text{g}$  of phosphate per 1 ml of eggs per min. Unfertilised eggs slowly release orthophosphate into sea water, while after fertilisation the diffusion of orthophosphate into the egg cells is achieved "against a large concentration gradient". Brooks, Chambers and White and many other scientists connect this phenomenon with stimulated synthesis of organic phosphorus compounds and with the adsorption of phosphate by the proteins of the dividing eggs.

This assumption gains support from the fact that after fertilisation of the egg cells there is a sharp rise in the demand for oxygen, consumption of which up to fertilisation is negligible (Tang Pei-Sung, 1931; Tyler and Humason, 1937; Horowicz, 1940; Borei, 1948, 1949; Zeuthen, 1949, 1950a, b, 1951, etc.). At the same time there is an increase in the exchange of carbohydrates, proteins and other compounds (Runström, 1933; Laser and Rothschild, 1939; Zielinski, 1939, etc.). Raising the temperature sharply increases the rate of absorption of orthophosphate by fertilised cells (Abelson, 1947). The penetration of ammonium chloride and alanine labelled with  $^{15}\text{N}$  and the inclusion of the  $^{15}\text{N}$  of these substances in the cell proteins is, according to Hultin (1953a, b, c), sharply enhanced on fertilisation of sea urchin eggs. Sharp increases in permeability for potassium and in the percentage of potassium exchanged by the eggs of marine animals (*Strongylocentrotus purpuratus* and *Arbacia punctulata*) occurs on their fertilisation (Chambers, White, Jeung and Brooks, 1948).

Unfertilised sea urchin eggs both with and without envelopes are permeable for radioactive calcium in exchange for the stable calcium of the eggs (Hsiao and Boroughs, 1958).

Kalman (1959) in experiments with unfertilised trout eggs showed that the water and sodium ions of the eggs are exchanged for labelled water and sodium from the surrounding medium, though the percentage exchange is not complete. The permeability of these eggs for chloride and iodide ions was also established.

It is interesting that live spermatozoa of the sea urchin *Arbacia* in sea water containing radioactive orthophosphate absorb radioactive phosphorus which is then included in the nucleic acids.

According to the observations of Cohn and Brues (1945), embryonic chicken muscle cells exchange all their potassium in 6 hr and their phosphate in 7 hr. Fenn, Noonan, Mullins and Haege (1941), experimenting with rabbits and frogs, and D'Silva and Neil (1951), using white rats, showed that radioactive potassium introduced into the blood of the animals very rapidly passes into the cells of the various tissues and organs.

According to Layton (1951) and Lowe and Roberts (1955), when sulphate ions labelled with  $^{35}\text{S}$  are introduced into chicken, rats, mice, guinea pigs and rabbits, radioactive sulphur is found in the cells of the various organs and tissues. Similar results are obtained in experiments on slices of the same organs when incubated for 4 hr in media containing radioactive sul-

phate. Rosenfeld and Tobias (1951) observed the inclusion in the cells of various organs of white mice of radioactive cobalt, copper and zinc, which were introduced intravenously in the form of cobalt, copper and zinc chloride. The same results were obtained by Uzunov *et al.*, (1959) on the introduction of radioactive cobalt into the blood of rabbits. Much data of this kind is assembled and presented in Hevesy's book (Hevesy, 1948).

Klein (1959) made a detailed study of the exchange of potassium and sodium ions of the earth amoeba *Acanthamoeba sp.* for the same ions from the medium. The amoeba were grown in an artificial nutritive medium. In the amoeba, their cysts and mitochondria the potassium and sodium concentrations and also the water content were determined. The results of these

TABLE 83. THE AMOUNT OF WATER, SODIUM AND POTASSIUM IN VEGETATIVE CELLS, CYSTS AND MITOCHONDRIA OF THE AMOEBA *Acanthamoeba sp.* (from Klein, 1959)

(The sodium and potassium concentrations are shown in mm/l of cell water, the water in per cent<sup>(1)</sup>)

	Water	Na <sup>+</sup>	K <sup>+</sup>	Ratio
Vegetative cells	80.4	32.4	57.5	1.74
Cysts	90.3	9.9	5.7	0.59
Mitochondria	69.2	28.9	25.1	0.87

<sup>(1)</sup> Diameter of amoeba about 20  $\mu$ ; mitochondria spherical with diameter about 0.5  $\mu$  and 1.0  $\mu$ ; cysts with diameter about 14  $\mu$ .

analyses are given in Table 83. It is characteristic that in the cysts and mitochondria the sodium concentration predominates over the potassium, whereas in the vegetative cells, as in the cells of multicellular organisms, the reverse is the case.

The process of the exchange of the amoeba sodium for  $^{24}\text{N}$  consists of two components: a fast component with a half time  $t_{\frac{1}{2}}$  of 5 min and a slow with  $t_{\frac{1}{2}} = 45-49$  min. It was established that, depending on the potassium concentration in the medium, only 35-50 per cent of the intracellular potassium is exchanged for  $^{42}\text{K}$  from the medium. The absolute rate of flux of potassium ions into the amoeba is 1.84 pm/cm<sup>2</sup> sec, and in the opposite direction 4.7 pm/cm<sup>2</sup> sec. (The experiments were performed with low potassium concentration in the medium.) The exchange of amoeba potassium also consists of two components: the fast component lasts 5-10 min. The fast phase covers 9 per cent of the cell potassium. This potassium, in a medium with a low potassium concentration, is released at a rate of 50-150 mm/hr: in the course of the subsequent 5 hr the release of potassium takes place at a rate of 1.5 mM/hr. The addition of calcium to the medium reduces the potassium ion flux into the cells and out of them, and also the rate of loss of potassium by the amoeba. The mitochondria of these amoeba occupy

20–40 per cent of the cell volume; they concentrate in themselves about 14 per cent of the cell sodium and 26 per cent of the cell potassium.

Thus, a very wide variety of cells of animal organisms, both *in vivo* and *in vitro*, is permeable for mineral anions and cations.

### 3. The Permeability of Plant Cells for Ions

As is well known, Overton (1896, 1899), on placing plant cells in solutions of various salts, came to the conclusion that they behave like typical osmometers and that the cells are completely impermeable for strong electrolytes. It is, however, important to bear in mind that it is penetration into the plant cell vacuole that is here under discussion. If the osmotic method indicates the inability of substances to penetrate the vacuole, this is not yet a proof that they do not penetrate the surface layers of the cytoplasm, because in certain cases the penetration of a substance into the vacuole can be prevented by its envelope—the tonoplast. Overton's conclusions were subsequently not supported by the numerous writers who used the same technique (Osterhout, 1909, 1911, 1912a, b, 1913; Fitting, 1915; Tröndle, 1918; Prat, 1922, 1923, and others).

Tröndle (1918), using the plasmometric method, showed that in order of decreasing rate of penetration into the cells of lupin roots cations and anions could be arranged in the following series: Rb, K, Na, Li, Mg, Ba, Sr, Ca and  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ .

Similar results were obtained by Pantanelli (1904) in experiments with mould fungi and by Ruhland and Hoffman (1925) with sulphur bacteria.

The permeability of plant cells for salts is proved not only by osmotic experiments (up to deplasmolysis) and the various modifications of this method, but also by direct chemical analysis of the contents of the plant cell vacuole after incubation in one or other salt solution. For these experiments a convenient subject is provided by certain algae whose cells have a large central vacuole. The contents of this last may be extracted and analysed. Osterhout (1922) used this technique to establish the permeability of the cytoplasm for  $\text{NaNO}_3$  and later he and his colleagues (Cooper, Dorcas and Osterhout, 1929) found by analysis of the cell juice of valonia that the chlorides of the alkali metals penetrate the vacuole of this cell, their rate of penetration varying as follows:



The same method also enabled Brooks (1922) to establish the permeability of hairweed cells for lithium, caesium and strontium chlorides.

A detailed study by Collander (1930, 1939) of the permeability of the giant cells of *Chara ceratophylla* and *Polipellopsis stelligera* for salts indicates that cations and anions penetrate through the cytoplasm into the vacuole in a great variety of conditions.

Particularly interesting are his experiments on the accumulation of lithium in the cell juice of the *Chara* algae with time. In weak solutions of lithium chloride the concentration of the cation of this salt in the juice turns out to be greater than in the surrounding medium, while in stronger solutions, there is, on the contrary, less. This process of lithium accumulation in the cell juice with time is shown in Fig. 89. Data on the dependence of the lithium concentration in the cell juice on its concentration in the medium are given in Table 84, from which it follows that in 63 hr there passes from a solution

TABLE 84. THE ACCUMULATION OF LITHIUM IN CELL SAP  
(from Collander, 1939)

Lithium concentration (in mequiv/l.)			Length of experiment (hr)
in medium (a)	in cell sap (b)	$\frac{a}{b}$	
0·05	0·29	5·80	63
0·50	1·40	2·80	63
5·00	3·40	0·68	63
50·00	6·50	0·13	63
0·1	0·62	6·200	124
1·0	1·90	1·900	124
10·0	3·70	0·370	124
100·0	8·80	0·088	124
0·1	0·58	5·8	165
1·0	2·40	2·4	165
10·0	5·30	0·53	165

containing 0·05 mequiv/l. of lithium into the cell juice a total of 0·29 mequiv/l., i.e., the concentration of this cation in the vacuole is 5·8 times greater than in the medium. From a medium containing 100 mequiv/l. of lithium, the passage of 124 hr sees only 8·8 mequiv/l. pass into the cell juice, i.e., the concentration of the cation in the cell vacuole is about one twelfth of that in the surrounding liquid.

Further, Collander showed that different cations and anions from equivalent solutions pass into the vacuole at different rates. By this criterion cations fall into the following series (in decreasing order):  $K^+$ ,  $Rb^+$ ,  $Li^+$ ,  $Cs^+$ ,  $Sr^{++}$ ,  $Mn^{++}$ . He also discovered that these cells can in certain conditions accumulate the above mentioned ions in considerable quantities.

The writer found that lithium is absorbed by the cells of the *Chara* algae in darkness 4–10 and in the light 4–15 times more slowly than rubidium; strontium 4–15 times slower than lithium; caesium always more slowly than lithium or rubidium. Cobalt is absorbed at the same rate as strontium. The

lithium ion is absorbed more rapidly than sulphate or thiocyanate, but more slowly than bromide.

Of great interest are Collander's data on the effect of oxygen and the hydrogen ion concentration on the accumulation of cations by plant cells. He established that the accumulation of lithium in the cell juice of algae in a

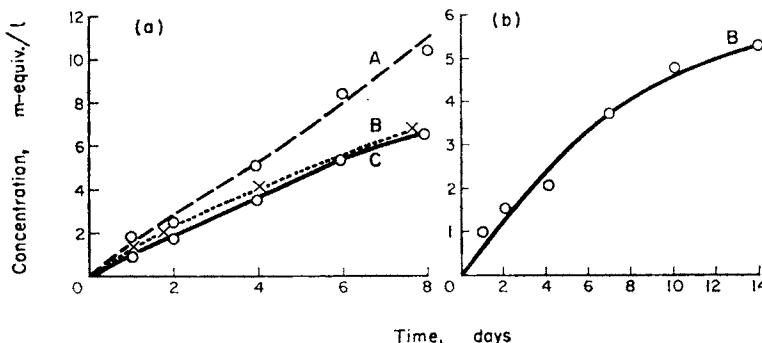


FIG. 89. The accumulation of lithium ions in the vacuoles of cells of *Chara ceratophylla* (in mequiv/l.) (from Collander, 1939).

a—in the medium (tap water) 50 mequiv/l. of lithium; b—in medium 1 mequiv/l. of lithium; A—in diffuse daylight; B—in a dark place; C—in a fully light-tight vessel.

nitrogen or oxygen atmosphere proceeds in exactly the same way. Experiments on the accumulation of lithium in the cell juice at various values of the pH of the medium (within the range pH = 5–8) showed that this process does not depend on the concentration of hydrogen ions. On the basis of these experiments, the author came to the conclusion that Osterhout's attempt (Osterhout, 1933) to explain the accumulation of cations in the cell vacuole by a sharp difference of pH between the vacuolar juice and the medium cannot be substantiated. Collander found no difference in principle in the absorption of ions by young (growing) and adult cells. Similar conclusions were reached by Sutcliffe (1954), who conducted some experiments on root sections of cucumber, maize and red cabbage. This cuts the ground from under the assertion of Höber (1945) that the difference in the ion concentration in the cell juice and the medium occurs only during the growth of the cells, this difference being subsequently maintained by the impermeability for salts of the membrane.

Hoagland, Hibbard and Davis (1928) in experiments with hairweed established that bromide can be accumulated in the cell juice out of dilute solutions in concentrations considerably exceeding the concentration of this anion in the surrounding medium. Diffusion equilibrium is attained when the bromide concentration in the cell juice reaches 5 mequiv at a concentration in the medium of 1 mequiv. The penetration of bromide into the cell juice leads

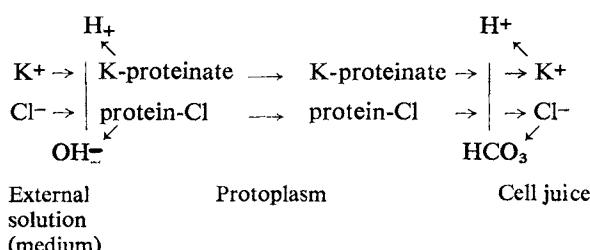
to the extrusion of chloride from the cell. The temperature coefficient of the accumulation of bromide in the cell juice is too high for diffusion and indicates the chemical nature of this process. Light energy is, as the authors show, the determining factor for bromide accumulation.

It is interesting that the ratio between the ion concentrations in the medium and in the cell sap is the same as the ratio of the concentrations in the liquid bathing the root of the plant and in the liquid of the growth vessels (in the xylem). This follows from data published in many papers (Tuyeva, 1926, 1928; Sabinin and Kolotova, 1927; Trubetskova, 1927; Sabinin, 1928; Bykov, 1929; Potapov, Solov'yeva and Ivanchenko, 1936, etc.).

Thus, Trubetskova (1927, 1940), experimenting with maize, found that the lower the concentration of calcium, potassium and phosphate ions in the medium, the greater the percentage of them relative to the external concentration that is found in the xylem. She suggests that this dependence of the ion concentration in the xylem is connected with the adsorption of them by the cells of the root which come into contact with the external solution.

Evidently, the adsorption of ions by the colloids of the protoplasm is a basic factor in the accumulation of ions by a plant cell, and their movement in the cell juice and in the growth vessels and also the extrusion of salts are connected with their desorption. This point of view is developed in numerous papers (Brooks, 1937; Sabinin, 1940a, b, 1955; Kolosov, 1940; Potapov, 1940; Trubetskova, 1940; Burström, 1948; Ratner, 1948, 1950, 1953 and many other writers). Lundegårdh too in a series of papers points out the importance of the role played by adsorption and desorption in the phenomena of absorption and desorption of mineral ions by plant cells (see his review: Lundegårdh, 1955).

According to Brooks (1947) the cations and anions which are formed in the course of metabolism in the cell can be adsorptively displaced by the cations and anions of the medium and thus be accumulated in the protoplasm and cell juice. This phenomenon is shown schematically by Brooks as follows:



Techniques for studying the permeability of cells for salts based on the use of radioactive isotopes have made it possible to demonstrate graphically the penetration of cations and anions into the protoplasm of plant cells and into the central vacuole. The data obtained by these techniques deserve

special attention because they have, for the most part, been obtained in physiological conditions.

Hevesy (1923) showed that the cells of bean roots that have been grown in a lead-bearing medium containing also its radioactive isotope (thorium-13) absorb this element. When plants containing the radioactive lead isotope are placed in a medium in which other cations are dissolved, the lead is observed to emerge from the cell at a greater rate than it does in distilled water (see Table 85).

TABLE 85. LOSS OF THE RADIOACTIVE ISOTOPE OF LEAD BY BEAN ROOT CELLS UPON IMMERSION IN  $10^{-2}$  N SOLUTION OF VARIOUS ELECTROLYTES  
(from Hevesy, 1923)

Nitrate solution	% of remaining radioactive lead in roots after immersion in nitrate solution
Lead (not radioactive)	5
Copper	3
Zinc	34
Cadmium	38
Chromium	43
Barium	74
Sodium	76

This table shows that, on penetrating the cell, the cations force out the intracellular lead and that this expressive force of the cations varies. The greatest amount of lead is forced out by copper and lead itself, the least by sodium.

Mullins and Brooks (1939) used the radioactive isotope method to study the penetration of potassium, sodium, rubidium and phosphorus into the protoplasm of hairweed cells. The seaweed was immersed for 15 min in a 0·01 M solution of radioactive salt. In this time the ion in question was found in the protoplasm, but not yet in the cell juice. After this the cells were washed in distilled water and then again immersed in 0·01 M solutions of inactive salts—the chlorides of lithium, sodium, potassium, rubidium and caesium; a Geiger-Müller counter was used to determine the release of the radioactive ion from the protoplasm. The authors found that the radioactive isotopes of potassium, rubidium, sodium and phosphate (and together with them also the same non-radioactive ions) penetrated at a noticeable rate into the protoplasm of hairweed cells. Potassium and rubidium penetrate more quickly than sodium. The reverse movement of the cation out of the cell, as was shown by the authors, obeys an exponential law.

Mullins and Brooks established that the rate of release of the cation from the cell depends on the cation content of the medium. This can be seen from the magnitude of the time constant  $K$  (see Table 86).

Experiments with radioactive rubidium isotopes gave the same results as those with labelled potassium, with only this difference that the rate of expulsion of rubidium from the cell by other cations was faster than for potassium. It follows from Table 86 that the release of potassium and sodium radioisotopes is lowest in distilled water.

TABLE 86. THE DISPLACEMENT OF THE POTASSIUM AND SODIUM IN THE PROTOPLASM OF HAIRWEED BY OTHER CATIONS

(from Mullins and Brooks, 1939)

Displacing cation	Magnitude of $K$ ( $\text{hr}^{-1}$ ) for	
	displaced potassium	displaced sodium
$\text{Li}^+$	0.12	0.33
$\text{Na}^+$	0.16	0.26
$\text{K}^+$	0.83	0.23
$\text{Rb}^+$	2.00	0.20
$\text{Cs}^+$	--	0.19
$\text{H}_2\text{O}$	0.05	0.05

In order of their ability to express potassium from the cells the basic alkaline cations can be placed, as the table shows, in the Hofmeister series, while the same cations express sodium from the protoplasm in the reverse order. The release of radioactive phosphorus from the cell is least also in distilled water and greatest in solutions of fluoride, chloride, iodide and bromide. The release of phosphate from the cell is achieved more quickly in a medium containing fluoride than in one containing iodide.

Similar results were obtained by Jenny and Overstreet (1938, 1939) and Jenny, Overstreet and Ayers (1939) in experiments on the accumulation of radioactive potassium by barley roots. The cells of barley roots that have been grown in earths containing the potassium radioisotope absorb a certain amount of labelled potassium. If the roots are subsequently placed in distilled water, no release of potassium by them is observed. If the roots are transferred to an earth or placed in a solution containing the chlorides of the alkaline or alkaline earth metals, then potassium is quickly released from the cells into the surrounding medium in exchange for the other cation. The release is greatest for the hydrogen ion and least for lithium. The bivalent cations of the alkaline earth metals have very little effect on the release of potassium from the roots. Competition between potassium and rubidium

is observed, and also between strontium and calcium in the process of the absorption of these cations by barley root cells. The absorption by these cells of lithium is strongly depressed by calcium. It is interesting that the absorption of potassium and rubidium is not only reduced in the presence of calcium, but is actually increased. The same effect is also produced by the presence in the solution of lithium (Epstein, 1960; and others).

Mazia (1938a, b, 1940) showed that water thyme cells which have absorbed calcium, strontium and barium cations do not release them into distilled water in 14 days; from this he draws the conclusion that the cations penetrating the cells are first bound by the proteins of the wall and only thereafter can they slowly pass into the protoplasm. If, however, water thyme cells are placed not in distilled water, but in solutions of potassium and sodium chloride, then they quickly lose both calcium and strontium and barium. On growing vetch in a solution of radioactive calcium Vlasyuk and Grodzinskii (1957) noticed that the cells of this plant absorb atoms of stable calcium in greater amounts than radioactive.

Brooks (1938a, b, 1939b, 1951) made a detailed study of the accumulation in hairweed protoplasm of the radioactive isotopes of potassium, sodium, rubidium, orthophosphate and bromide. The permeability of the protoplasm for these cations falls in the following series: rubidium  $\geq$  potassium > sodium. Brooks found that these cations and bromide quickly pass into the cell protoplasm and very slowly into the central vacuole, which indicates, in the author's opinion, the special properties of the cell cytoplasm. The nature of the accumulation of sodium in the protoplasm was observed to be the same by Mullins (1939).

Brooks (1940) showed that if spirogyra are placed for a total of 15 sec in a solution of sodium chloride with the addition of radioactive sodium, then their protoplasm already reveals a high degree of radioactivity. It is important to notice that what occurs here is not adsorption of sodium by the wall, but absorption by the cortical layer of the protoplasm.

MacRobbie and Dainty (1959), experimenting with the algae *Nitellopsis obtusa*, found that the potassium ion flux out of the wall into the cytoplasm is equal to 4 and from the cytoplasm into the vacuole  $0.25 \text{ pm}/\text{cm}^2 \text{ sec}$ . The potassium ion flux from the medium into the wall is considerably higher than these values. Diamond and Solomon (1959) studied the exchange of intracellular potassium from the fresh water algae *Nitella axillaris* for potassium from the surrounding medium with the aid of radioactive potassium. These cells maintain the intracellular potassium concentration on a level about  $0.13 \text{ M}$ , which is about 2000 times greater than in the surrounding medium. By measuring the change in specific radioactivity with time of whole cells and also of isolated cellulose walls, cytoplasm and vacuolar juice, these authors were able to establish that the process of the exchange of cell potassium for medium potassium consists of three components, corresponding to the rate of exchange of potassium from the wall for potassium

from the medium, of potassium from the cytoplasm (including the chloroplasts) for potassium from the walls and potassium from the cytoplasm for potassium from the vacuole. The half-time for potassium exchange for the walls is 23 sec, for the cytoplasm 5 hr and for the vacuole 40 days. The total cell potassium was distributed among the components as follows: in the cellulose wall about 0·1 per cent, in the cytoplasm 1·6 per cent and in the vacuole 98 per cent. The potassium ion fluxes are: from the medium into the cell wall 50, from the envelope into the cytoplasm 0·47 and from the cytoplasm into the vacuole 0·72 pm/cm<sup>2</sup>sec.

Hayward and Scott (Hayward and Scott, 1953; Scott and Hayward, 1954) showed that the cells of the marine green algae *Ulva lactuca* lose up to 80–90 per cent of their sodium and potassium if the algae are kept in an isotonic sucrose solution. If the cells are then placed in sea water, the potassium is completely reaccumulated. This process is independent of the presence of sodium ions in the medium. The accumulation of potassium and the secretion of sodium by the cells of the algae are depressed by the action of a number of inhibitors. Similar results were obtained in experiments with another type of marine algae—*Valonia macrophysa*.

The cells of the red sea algae *Porphyra perforata*, like many live cells, contain a high potassium concentration and a relatively low concentration of sodium and chloride by comparison with the concentration of these ions in sea water. This ion distribution is maintained by the cell metabolism. If the algae are kept in sea water without potassium the cells lose this ion and absorb sodium. When kept in sucrose solution, the cells release potassium and sodium, the latter being replaced more rapidly than the former. When impoverished of potassium, the cells can accumulate rubidium ions if placed in sea water in which the potassium ions have been replaced by rubidium (Eppley, 1958a, b).

A large volume of work has been published to date in which the mineral exchange of micro-organisms, especially yeast cells, in various conditions has been comprehensively investigated. Detailed reviews of the literature on the problem are to be found in several books and articles (Hinshelwood, 1946; Conway, 1954, 1955; Rothstein, 1954, 1955, 1959, 1960, 1961; Mitchell, 1957, 1959; Mitchell and Moyle, 1956; Harris, 1960 and others).

Like other animal and plant cells, micro-organisms have as their basic cation potassium with various phosphorus compounds as principal anion. Rothstein (1955) gives the following data as characterising the "ion" composition of baker's yeast (in mm/kg of wet weight of cells): K<sup>+</sup>—170, Mg<sup>++</sup>—20, Ca<sup>++</sup>—3, Mn<sup>++</sup>—0·2, HCO<sub>3</sub><sup>-</sup>—50, total phosphorus—120, acid-extractable phosphorus—40, orthophosphate—15, succinate—7, ether-soluble acids—15. Some authors suppose that the great mass of the bivalent cations is present in the cells in the bound state with phosphate groups of the nucleic acids and with inorganic polyphosphates. On the other hand, the potassium ions are in the free state and their electrical charges are balanced

by the  $\text{HCO}_3^-$ , by the anions of the organic acids, the orthophosphate and the phosphoric esters. This conclusion about the freedom of the potassium is reached on the basis of plasmolytic experiments with micro-organisms which indicate the high osmotic pressure of the contents of these cells (about 11–20 atm, see references Rothstein, 1959, 1960; Mitchell and Moyle, 1956).

Hill (1932) and Collander (1956), using the change in the intensity of brightness of luminescent sea bacteria as an indicator of the degree of their plasmolysis, came to the conclusion that these organisms behave like osmometers and that they are permeable to sucrose and the following tested salts:  $\text{NaCl}$ ,  $\text{NaNO}_3$ ,  $\text{KCl}$ ,  $\text{LiCl}$ ,  $\text{MgSO}_4$ ,  $\text{NaBr}$ ,  $\text{KI}$ ,  $\text{NaHCO}_3$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{NaSO}_4$ ,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{K}_2\text{SO}_4$  and  $\text{SrCl}_2$ .

Many writers have concluded that microbial cells are not permeable for electrolytes, also on the basis of results obtained in studying the distribution of salts between the micro-organisms and the medium. In a number of cases these experiments show that the concentration of the electrolyte and of the individual ions that penetrate the cell, as calculated in relation to the cell water, is considerably lower than their concentration in the surrounding medium. Similar facts form the basis of the conclusion that electrolytes pass only into the cell envelope and do not penetrate the cell (protoplasmic) membrane into the interior of the cell.

However, the experimental data on which this conclusion is based is remarkable for its extreme contradictoriness. Thus, Paine (1911), studying the permeability of the yeast cell for sodium chloride, ammonium sulphate and sodium arsenate, found that in equilibrium conditions the concentration of these salts in the cells was always 5–6 times lower than in the surrounding medium. According to Mitchell and Moyle (1956) *E. coli* have only 10 to 12 per cent of their volume available for the penetration of phosphate, whereas according to MacDonald and Gerhardt (1958), the volume available for the penetration of sulphate is 35 per cent in these cells.

Rothstein (1955, 1959, 1960, 1961), on analysing the results of a large number of workers, came to the conclusion that yeast cells and other micro-organisms are not permeable by free diffusion for anions (phosphate, chloride, sulphate, pyruvate, citrate, succinate) and for bivalent cations ( $\text{Mg}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{UO}_2^{++}$ ), but that they do have this property for univalent cations, only in a limited degree. The penetration of anions and cations into these cells is seen as an exchange diffusion process with the participation of membrane carriers. This point of view is shared by a number of other workers too (Mitchell, 1953, 1954a, b, 1957, 1959; Mitchell and Moyle, 1956; Conway, 1954, 1955 and others).

During the fermentation of sugars by micro-organisms in the presence of the medium of potassium salts a considerable accumulation of this cation occurs accompanied by the release from the cells into the medium of an equivalent amount of hydrogen ions. This phenomenon has been demonstrated in experiments with yeast cells (Pulver and Verzár, 1940; Conway

and O'Mally, 1946; Conway and Dawney, 1950a, b; Conway and Brady, 1950; Conway, Brady and Carton, 1950; Rothstein and Enns, 1946; Rothstein and Demis, 1953; Rothstein and Bruce, 1958a; Kometiani, 1956, 1960 etc.) and with bacteria (Hinshelwood, 1946; Eddy and Hinshelwood, 1950; Roberts and Roberts, 1950 and others).

When sugar is fermented in the presence of potassium chloride the yeast cells can accumulate up to 0·3 M potassium per kg of wet weight of cells, and the pH of the medium can drop during this process as low as 1·4. The potassium concentration in the cells can be 1000 times greater than in the medium (Pulver and Verzár, 1940; Conway and O'Malley, 1946; Conway and Dawney, 1950a, b; Rothstein and Bruce, 1958a, b and others), so that the passage of potassium into the cells and the release of hydrogen ions from them goes "against" a considerable concentration gradient (if we take into account that the potassium and hydrogen ions in the cells are in the free form). Besides potassium, phosphate and other cations and anions, these cells can in certain conditions accumulate ammonium (Conway and Breen, 1945) and utilise mineral sulphate (see Kleinzeller, Kotyk and Kováč, 1959; Kotyk, 1959).

The potassium accumulated by yeast cells is slowly exhausted when they are washed with potassium-free solution. At 25° the cells lose into a potassium-free medium 8–10 mm of potassium per kg of cells per hr. Lowering the temperature leads to a considerable reduction in the rate of loss of potassium (Rothstein and Bruce, 1958a, b).

Ions such as those of rubidium, sodium and lithium can also penetrate yeast cells in exchange for hydrogen ions, but at a lower rate than the exchange process for potassium ions (Conway and Duggan, 1958). It has been shown that inorganic cations and many organic ions (basic amino acids, ethylamine) can compete in the process of exchange of cations from the cells for cations from the medium; in order of decreasing competitive power, the following series is obtained: K, Rb, Cs, Na, Li = Mg. It is interesting that this series of competing cations is also obtained in experiments with resting (non-fermenting) cells (Conway and Beary, 1958).

A number of workers have used radioactive potassium to study the exchange of the potassium of micro-organisms for potassium of the surrounding medium. It has been shown that the speed of this process depends on temperature, the potassium concentration in the medium and in the cells and also on the pH and the presence of other cations in the medium. The rate of exchange of cell potassium for  $^{42}\text{K}$  from the medium is significantly higher in fermenting cells than in resting (Hevesy and Nielsen, 1941; Rothstein and Bruce, 1958a; Rothstein, 1960c, and others). The sodium of micro-organisms is exchanged at a rapid rate for  $^{24}\text{Na}$  (Cowie, Roberts and Roberts, 1949; Roberts and Nieset, 1950; Conway and Hingerty, 1953). During fermentation the bacteria can accumulate, besides phosphate, both potassium and rubidium or caesium ions (Eddy *et al.*, 1950).

Non-fermenting yeast cells can preserve their high potassium concentration constant for a long time if the potassium concentration in the medium is  $5 \times 10^{-5}$  M. In these conditions the potassium ion fluxes into and out of the cell are equal. If the potassium concentration in the medium is lower than this value, the potassium ion flux into the cells is lower than in the opposite direction, and in this case the potassium concentration in the cells falls. If the potassium concentration in the medium is higher, the reverse phenomenon occurs. Raising the hydrogen ion concentration in the medium leads to loss of potassium by the cells, the potassium ion flux out of the cells exceeding the flux in the opposite direction (Rothstein and Bruce, 1958 a).

Rothstein and Bruce (1958 a) showed that the rate of absorption of potassium by baker's yeast changes in dependence on the potassium concentration in the medium in accordance with the Michaelis-Menten rule. The same was found by Conway and Duggan (1958) for the rate of absorption by fermenting cells of potassium, rubidium, sodium and magnesium. These authors established that the maximum rate of absorption is greatest for potassium and least for magnesium ( $K^+$ ,  $Rb^+$ ,  $Na^+$ ,  $Mg^{++}$ , in descending order).

Conway and his colleagues (Conway and Hingerty, 1953; Conway and Moore, 1954; Conway, Ryan and Carton, 1954) showed that baker's yeast in a potassium-free medium with a high sodium ion content can accumulate large amounts of sodium in exchange for the hydrogen ions that are formed in the cells during the fermentation of glucose. Thus, for a sodium nitrate concentration in the medium of 0.2 M in the presence of 5 per cent glucose yeast cells accumulate up to 140 mequiv/kg of sodium, while their potassium concentration drops from 140 to 8 mequiv/kg. When such cells are washed with tap water they lose up to 30 per cent of their sodium in 2 hr, and up to 40 per cent in 4 hr. In this case the release of sodium from the cells is achieved together with an equivalent quantity of anions (bicarbonate, succinate, and others: see Conway and Moore, 1954). If yeast cells with a high sodium content are placed in a medium containing potassium ions, then potassium is accumulated in exchange for the cell sodium. Replacement of cell sodium by potassium also occurs when yeast cells containing equal amounts (80 mm/kg) of potassium and sodium are placed in a medium with 0.1 M KCl + 0.1 M NaCl (Conway, Ryan and Carton, 1954). In this case the passage of potassium into the cells is accomplished not only in exchange for sodium, but also partially in exchange for hydrogen ions (Foulkes, 1956). Kotyk and Klein-zeller (1959) discovered that loss of sodium by yeast cells is increased on incubation of the cells in hypertonic solutions of fermenting and non-fermenting sugars. The loss of sodium by the cells in these conditions occurs together with their dehydration.

The permeability of the cells of micro-organisms for bivalent cations has been studied by a number of workers (Schmidt, Hecht and Thannhauser, 1949; Rothstein and Hayes, 1956; Rothstein, Hayes, Jennings and Hooper, 1958; Jennings, Hooper and Rothstein, 1958; Conway and Beary, 1958;

Rothstein, 1961; Netter and Sachs, 1961 and others). According to these writers, non-fermenting yeast cells are completely impermeable for bivalent cations. They can be adsorbed on the surface of the cells. This process is reversible and reaches complete saturation in a few minutes. Cations form the following series in order of decreasing ability to be adsorbed:  $\text{UO}_2^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{Mg}^{++}$  (Rothstein and Hayes, 1956).

However, fermenting cells in the presence of potassium and orthophosphate absorb bivalent cations in large amounts together with potassium and phosphate. In this case the rate of penetration of the cations into the cells falls in the reverse order, that is:  $\text{Mg}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{UO}_2^{++}$  (Rothstein, Hayes, Jennings and Hooper, 1958). According to Rothstein (1960, 1961; Jennings, Hooper and Rothstein, 1958) the passage of bivalent cations into cells is accomplished by means of membrane carriers which are synthesised during the fermentation of the sugars in the presence in the medium of potassium and phosphate. Arsenate depresses the absorption by the cells both of phosphate and of bivalent cations ( $\text{Mn}^{++}$ ).

According to Rothstein, the carriage into the cells of bivalent cations is accomplished by specific carriers, but in the opinion of Conway and his colleagues (Conway and Beary, 1956), magnesium ions are transported into yeast cells by the same carriers as potassium.

The permeability of plant cells for phosphate in various conditions has been studied with the phosphorus radioisotope by many workers; as a result it has been firmly established that phosphate penetrates not only into the protoplasm of cells, but also into the cell vacuole (Hevesy, Linderstrom-Lang and Olsen, 1936, 1937; Mullins and Brooks, 1939; Klechkovskii, Ivanenko, Bagayev and Rachinskii, 1947; Hevesy, 1947; Klechkovskii and Yevdokimova, 1951; Klechkovskii, Stoletov and Yevdokimova, 1951; Klechkovskii, Tselishchev and Yevdokimova, 1951 and others).

An enormous amount of work has been devoted to the study of the permeability of micro-organisms for phosphate. The results of this work are discussed in detail in review articles and books by various writers (Kamen and Spiegelman, 1948; Mitchell, 1954a, 1957; Mitchell and Moyle, 1956; Rothstein, 1954, 1955, 1956, 1959, 1960, 1961; Hinshelwood, 1946; Harris, 1960). We cite here only a selection of the experimental data.

It has been shown that resting (non-fermenting) cells of bacteria and yeast can very slowly and in small amounts absorb mineral phosphorus from the surrounding medium and slowly lose phosphorus when washed (Mullins, 1942; Hevesy and Zerahn, 1946; Juni, Kamen, Reiner and Spiegelman, 1948; Mitchell, 1953, 1954c; Hotchkiss, 1943; Rothstein, 1955; Goodman and Rothstein, 1957). They also slowly exchange intracellular phosphorus for radioactive phosphorus from the medium (Hevesy, Linderstrom-Lang and Nielsen, 1937; Mullins, 1942; Goodman and Rothstein, 1957).

Rapid absorption of external phosphate by the cells of micro-organisms occurs only when the specific substrate which they ferment is present in the

medium and also cations of potassium, magnesium and certain others which are also absorbed in large quantities by the cells. Thus, in experiments with the bacteria *M. Pyogenes* (Mitchell, 1953), *S. aureus* (Hotchkiss, 1943) and yeast cells (Goodman and Rothstein, 1957) it was shown that the absorption by the cells of phosphate proceeds at the greatest rate if there is glucose present in the medium. If the glucose is replaced by another substrate which is also fermented by the cells (lactate, acetate, pyruvate etc.), then the absorption of phosphate is completely stopped or, in certain cases, sharply depressed. The absorption of phosphate is depressed by certain poisons which suppress glycolysis (Kamen and Spiegelman, 1948; Spiegelman, Kamen and Sussman, 1948; Mitchell, 1954b; Rothstein, 1960, 1961). All this indicates that the absorption of phosphate by micro-organisms is a specific process which is connected with the glycolytic cycle. It has been shown in experiments with bacteria and yeast cells that, parallel to the absorption of phosphate, absorption is also observed to take place of cations of potassium, rubidium, caesium, magnesium, manganese, calcium and, in special conditions, of sodium and lithium (Eddy *et al.*, 1951; Rothstein, 1959, 1960, 1961 etc.).

The rate of absorption of mineral phosphorus by bacteria (Mitchell, 1953, 1954a; Mitchell and Moyle, 1956) and by yeast cells (Goodman and Rothstein, 1957) changes depending on the phosphate concentration in the medium on the adsorptional pattern (according to the Michaelis-Menten rule) just as is observed in the case of the absorption by the cells of potassium, manganese and the other cations mentioned above.

Thus, the maximum rate of adsorption of phosphate by baker's yeast is about 160 mm/kg of yeast cells per hr at a pH of 4·0 and an external potassium concentration of 20 mequiv/l. (Goodman and Rothstein, 1957).

The mechanism of the penetration of the anion of phosphoric acid into the cells of micro-organisms, as also in the case of other animal and plant cells, has not yet been clarified. The literature contains discussions of two possible paths for the penetration of this anion into the cells of micro-organisms: simple diffusion through the cell surface with subsequent inclusion of the phosphorus in organic phosphorus compounds or esterification of mineral phosphorus in the cell membrane and its transport into the cell by membrane carriers with the participation in the process of a complex enzymatic mechanism (Kamen and Spiegelman, 1948; Mitchell, 1954a, b, 1957; Rothstein, 1955, 1956, 1959, 1960, 1961 etc.).

Thus, plant cells, like animal cells, are freely permeable for all mineral anions and cations; live cells have no property of selective permeability for mineral ions.

The rate of absorption of mineral ions by any cell, and also the distribution of these ions between the medium and the live matter, is closely connected with metabolism and is in the last analysis completely determined by the enzymatic reactions which take place in the cell. This question will be examined in detail in a separate chapter.

*4. Conclusions*

1. Animal and plant cells have no selective ionic permeability; they are permeable for all mineral cations and anions.

2. In plant and animal cells it is characteristic that there is an inequality of the concentrations of the individual mineral ions in the cell and the medium. The concentrations of potassium, magnesium, calcium and phosphorus in the protoplasm are many times greater than in the medium, whereas the concentrations of sodium and chloride, on the other hand, are several times greater in the medium than in the protoplasm.

3. A slight reduction in the concentration of the potassium, sodium, chloride and other ions in the medium leads to their release from the cell, the diffusion of sodium and chloride outwards being achieved "against the concentration gradient". When the concentration of these ions in the medium is increased, they are observed to pass into the cell, and in this case it is the diffusion of potassium that takes place "against the concentration gradient".

4. The facts noted in paragraphs 1-3 above are fundamentally contradictory to the conclusions of the classical membrane theory and are completely inexplicable from the standpoint of that theory.

## CHAPTER IX

# The Mechanism of the Distribution of Mineral Substances between the Cell and Medium

### 1. *The Membrane Theory of the Distribution of Mineral Substances between the Cell and Medium*

At the beginning of the preceding chapter certain data were quoted which indicate that live cells contain a high concentration of potassium, mineral phosphorus and certain other ions which are present only in low concentrations in the medium, while the reverse ratio of concentrations holds for sodium and chloride ions. There is not at present any generally accepted theory for explaining this phenomenon.

*The Donnan Principle of the distribution of mineral ions.* One theory widely recognised among scientific workers is that of Boyle and Conway (1941). According to this theory, the distribution of mineral ions between resting muscle cells and their medium is determined by Donnan membrane equilibrium. In building up this theory, the authors started from the following propositions: (a) the osmotic pressure inside a fibre is equal to the osmotic pressure of the medium; (b) there are present in the fibres large amounts of non-diffusible substances which cannot pass through the protoplasmic membrane, such as proteins, the esters of phosphoric acid, coenzymes of one sort and another and so on; (c) the protoplasmic membrane is a porous formation whose pores are freely permeable only for those mineral anions and cations whose radius (together with their hydrate shell) is not greater than the radius of the potassium ion ( $H^+$ ,  $Rb^+$ ,  $Cs^+$ ,  $NH_4^+$ ,  $K^+$ ,  $OH^-$ ,  $Br^-$ ,  $I^-$ ,  $Cl^-$ ,  $NO_3^-$ ) and is completely impermeable to all ions with a greater radius than this ( $Na^+$ ,  $Li^+$ ,  $Ca^{++}$ ,  $Mg^{++}$ ,  $CH_3COO^-$ ,  $SO_4^{--}$ ,  $HPO_4^{--}$ , etc.); (d) the number of negative charges which are carried by the non-diffusible substances should be balanced by the cations which are able to penetrate the cell.

On the basis of these propositions they proceeded to deduce the following fundamental equations of the membrane theory (Boyle and Conway, 1941; Conway, 1946, 1947, 1957, 1960a):

For osmotic equilibrium —

$$\eta/v = C - (b_i + d_i), \quad (1)$$

where  $\eta$  is the molar (osmolar) concentration of non-diffusible substances in a cell;  $C$  the external molar (osmolar) concentration,  $b_i$  and  $d_i$  the intra-

cellular concentration of diffusible cations and anions (univalent diffusible anions and cations are taken by way of example);  $v$  the volume of cell water, taken as unity.

For electrical neutrality within a cell –

$$E/v = b_i - d_i, \quad (2)$$

where  $E$  is the electrically negative charges carried by the non-diffusible substances in the cell.

For Donnan equilibrium to apply to mineral ions that pass passively into and out of a cell in a state of equilibrium (i.e. when the ion flux into the cell is equal to the flux from the cell into the medium) the following relation must hold: –

$$b_i \times d_i = b_o \times d_o \quad (3)$$

where the subscripts  $i$  and  $o$  refer to the inside and outside of the cell respectively.

From these equations it follows, for example, that for isolated frog sartorius muscle (where  $\eta$  is practically the same as  $E$ , Conway, 1957), the concentration in the muscle fibres of potassium  $[K]_i$  will be determined by the formula

$$[K]_i = \frac{1}{2}C - (\eta - E)/2v, \quad (4)$$

and of chloride ions –

$$[Cl]_i = 2([K^+]_o \times [Cl^-]_o)/C, \quad (5)$$

and generally, the concentration of anions which penetrate a cell is given by: –

$$d_i = \frac{1}{2}C - (\eta + E)/2v. \quad (6)$$

The amount of water in a cell  $v$  will be determined by the equation –

$$v = (\eta + E)/2[Na^+]. \quad (7)$$

It also follows from this theory that passively penetrating cations and anions that are present in the medium (in blood plasma, Ringer's solution, etc.) should be distributed in inverse proportions in conditions of stationary equilibrium: for example:

$$[K]_i/[K]_o = [H]_i/[H]_o = [Cl]_o/[Cl]_i. \quad (8)$$

It was shown by the authors of this theory that when the potassium chloride concentration in the medium is raised above 10 mequiv/l, potassium passes together with chloride ions into the muscle fibres. In this case the muscles swell.

*The hypothesis of the active transport of ions and the sodium pump.* It is intelligible that equation (4) will be valid if sodium ions are absolutely incapable of passing through the cell membrane, just as equations (5) and (6) can hold if the other anions of the medium ( $HCO_3$ , orthophosphate) are not

able to penetrate the cell. However, after the well-known work of Heppel (1939), whose results, as we have seen, were confirmed by many other workers (see preceding chapter), it has become clear that sodium ions are capable of penetrating a great variety of cells, including both nerve and muscle fibres. Therefore the Donnan membrane principle of the distribution of mineral ions between a cell and its medium is not supported by the evidence.

A way out of the difficulty with which the membrane theory was confronted was soon proposed by Dean (1941). He suggested that sodium ions first pass into a cell passively, along the concentration gradient, but that they are then actively expelled (pumped out) of the cell by means of a special membrane mechanism which has been called the "sodium pump". This pump does work ensuring the movement of sodium ions out of a cell into the medium against the concentration gradient. The energy for the work of the sodium pump is supplied by the metabolic reactions of the cell.

This hypothesis of the sodium pump has frequently been brought in to explain facts obtained in experiments with muscles (Conway and Hingerty, 1948; Conway, 1957, 1960a, b; Carey, Conway and Kernan, 1959; Conway and Mullaney, 1961; Conway, Kernan and Zadunaisky, 1961; Steinbach, 1961 etc.), with nerves and muscles (see review: Hodgkin, 1951, 1958), with cortical slices from mammalian kidneys (Mudge, 1951; Conway and Geoghegan, 1955; Cort and Kleinzeller, 1957, 1958; Kleinzeller and Cort, 1957a, b, 1960; Kleinzeller, 1960, 1961), with yeast cells (Conway, Ryan and Carton, 1954), with erythrocytes (Maizels, 1961). Ussing (1953) reviewed a large number of facts with the aim of clarifying the applicability of the sodium pump to the cells of various plants.

The ideas of the promoters of this hypothesis of the sodium pump is that the pump works to create conditions such as would hold if no sodium ions penetrated into a cell. In this case it is possible to apply the Boyle-Conway theory to explain the asymmetrical distribution of mineral ions between a cell and its medium.

Proposals about the working mechanism of the sodium pump have been made by several workers in numerous papers. Thus, Conway (1951, 1953a, b, 1957, 1960a, b) put forward the hypothesis of an oxidising-reducing mechanism for bringing sodium ions out of a cell against the concentration gradient. His idea is that the metal ions of the oxidising-reducing (cytochrome) system by which electrons are transferred to oxygen act as carrier and energy source for the active transport of sodium ions from a cell. One component of this system acts as an electron donor, the other as acceptor. The electron donor is essentially the carrier of the sodium from a cell into the medium: on the inner side of the membrane the sodium ion fastens onto it, while the donor on the outside of the membrane gives up an electron to the second component of the oxidising-reducing system and the sodium ion is accordingly released into the surrounding medium. This active transport of sodium

out of a cell produces the state of affairs whereby the accumulation of potassium ions in a cell follows the Donnan principle.

The Cambridge school of physiologists, led by Hodgkin, also uses the Boyle-Conway theory to explain the asymmetry of the mineral ion distribution between a cell and the medium with the addition of the sodium pump hypothesis (Hodgkin, 1951, 1958).

Hodgkin (1958) assembled a vast quantity of facts which indicate that, in certain experimental conditions, for every sodium ion brought out of a cell there is one potassium ion that enters the cell, and that many factors, for example, certain exchange inhibitors, which depress the removal of sodium from the cell, also stop the accumulation of potassium in the cell. In these conditions it is frequently found that potassium and sodium ions move along the concentration gradient in equivalent quantities: the sodium into the cell and the potassium out of the cell into the medium. This phenomenon was discovered in experiments with muscles (Steinbach, 1952; Desmedt, 1953; Keynes, 1954; Edwards and Harris, 1957), with erythrocytes (Harris and Maizels, 1951; Shaw, 1954; Glynn, 1956), with leucocytes (Wilson and Manery, 1948, 1949; Hempling, 1953, 1954) and with other tissues.

Observations of this kind have been taken as grounds for supposing that the extrusion of sodium from a cell and the uptake of potassium by it are linked processes and that, possibly, the same membrane carriers are the agents whereby the processes are realised. A possible physico-chemical basis for such a system has been discussed by a number of authors (Harris, 1954; Shaw, 1954; Hodgkin and Keynes, 1954; Glynn, 1956; Davis and Keynes, 1961).

It is supposed (see Hodgkin and Keynes, 1955a; Hodgkin, 1958) that a membrane carrier ( $x$ ) on the outside of the membrane has a specific affinity for potassium with which it forms an undissociated compound ( $Kx$ ) and diffuses to the inside of the membrane. Here the carrier ( $x$ ) changes chemically, loses its affinity for potassium and turns into a carrier ( $Y$ ) which now has a specific affinity for sodium, forming the complex ( $NaY$ ). This complex diffuses to the outside of the membrane where ( $Y$ ) again turns into the carrier ( $x$ ), releases the sodium and joins with potassium, carrying it into the cell. If the undissociated compounds  $Kx$  and  $NaY$  are capable of traversing the membrane more easily than in the dissociated form, then the system should act as a neutral pump with one sodium ion being removed in exchange for the absorption by the cell of one ion of potassium. It is supposed that the energy necessary for the operation of such a pump is consumed in the process of the conversion of the potassium carrier ( $x$ ) into a sodium carrier ( $Y$ ).

Several writers have done work which shows that the energy source for the sodium pump can be found in the decomposition of certain energy-rich phosphorus compounds such as ATP (Caldwell, 1956, 1957, 1960; Caldwell and Keynes, 1957; Keynes, 1961; Caldwell *et al.*, 1960a, b).

The accumulation by plant cells and micro-organisms of mineral substances and also the asymmetric distribution of individual ions between cells and medium is at present considered by the majority of scientists from the standpoint of active transport with the participation in some cases of specific pumps. The abundant literature on the subject has recently been summarised in articles by various authors (Lundegårdh, 1955; Sutcliffe, 1959; Ussing, 1953; Conway, 1954, 1955; Mitchell, 1961; Rothstein, 1959, 1960, 1961; Robertson, 1960).

In connection with Conway's oxidising-reducing (redox) hypothesis of the sodium pump, it should be noted that a similar hypothesis had been put forward considerably earlier by Lundegårdh (1939) to explain the mechanism of the accumulation of salts by plant cells. This hypothesis was based on the fact that increases in the accumulation of salts by plant cells are tied up with increases in their oxygen requirements — a phenomenon called anion (Lundegårdh and Burström, 1933) or salt (Steward, 1937; Robertson, 1941) respiration. There is a definite quantitative relation between the rate of anion (salt) respiration and the rate of accumulation of salts: factors which promote salt respiration also increase the rate of accumulation of salts, while several inhibitors of anion (salt) respiration also depress the accumulation of salts in cells. This gave Lundegårdh (1939) grounds for supposing that the cytochrome system which acts as an electron carrier in the direction of the outside surface of cells can also operate as an anion carrier in the reverse direction (from the outside of the membrane into the cell). This is the active pathway for anion movement. Besides this there is also a passive (adsorptive) pathway which, with the aid of the membrane carriers, ensures the movement of cations into the cell along the electrochemical gradient. This pathway can also be used for the exchange of the hydrogen ions formed in the course of metabolism in the cell for the external mineral cations of the medium (Lundegårdh, 1939, 1940, 1945, 1946, 1947, 1954, 1955, 1960).

The difference between Conway's hypothesis and Lundegårdh's consists in this, that, according to the former, the cytochrome system of the cell membrane effects active transport of cations (sodium), while, according to the latter, the same system effects active transport of anions into the cell. Sutcliffe (1954) also asserts that it is not anions but cations that move actively into a plant cell.

If there really is a system controlling the active transport of ions in plant cells, the question of its localisation remains in dispute: is it in the protoplasmic membrane, in the cytoplasm, or is this function performed by the surface layer of the protoplasm as a whole or, possibly, by the mitochondria in it? It is at present impossible to answer this question. The trouble is that the accumulation of mineral ions takes place not only in the cytoplasm of a plant cell, but also in its vacuolar juice (see Krogh, 1946; Robertson, 1959).

*Critique of the hypothesis of membrane pumps.* In recent years several writers have brought forward serious arguments against the hypothesis of

the sodium pump and in general against theories of a membrane mechanism for the active transport of substance into a cell and out of the cell into the medium (Ling, 1952, 1955; Dervichian, 1955; Heilbrunn, 1956; Ungar, 1957; Shaw and Simon, 1955a, 1955b; Simon, 1959, 1961; Ernst, 1958; Nasonov, 1959a; Kurella, 1960, 1961; Liberman and Chailakhyan, 1961 and others).

Dervichian (1955) made a detailed examination of the available hypotheses and facts relating to the permeability of cells, as a result of which he came to the conclusion that the existence of a membrane with the peculiar properties that had to be ascribed to it to explain selective permeability and the selective accumulation of substances within cells could have no biological

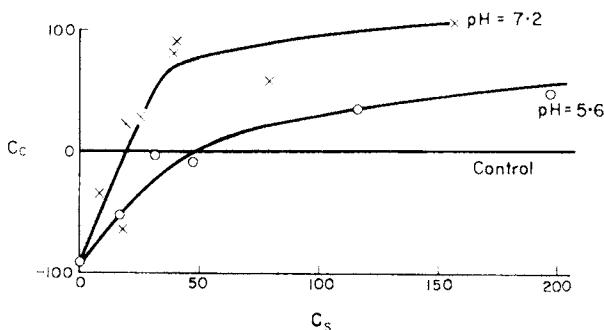


FIG. 90. The dependence of the potassium concentration in frog muscles ( $C_c$ ) on the concentration of this cation in the medium ( $C_s$ ) at various pH (in mg percent) (from Fenn and Cobb, 1934/5).

or biochemical foundation. Further, the author points out that, even if we consider that the existence of a cell membrane has been proved morphologically, all the same it is extraordinarily difficult to imagine how it can be selectively permeable, this property change depending not only on the nature of the substances, but also on the direction of their motion, whether into or out of a cell. Nasonov (1959a) discusses this subject in his book.

In fact, if the modern membrane theory is taken to its logical conclusion, it is necessary to assume the existence in the cell membrane of an innumerable multitude of different kinds of specific pumps regulating the passage and distribution not only of individual mineral and organic ions, but also of non-electrolytes, both those substances which a cell uses constantly and those which it encounters only in experimental conditions. I cite here only a few examples which, in my view, place the modern membrane theory of the distribution of mineral substances in a difficult position.

As we have seen, the potassium concentration in frog muscle fibres is about 50 times greater than in the medium. According to the Boyle-Conway

theory, hydrogen ions, like potassium ions, in agreement with the Donnan principle, should be distributed between a cell and the medium in the same proportions:

$$[K]_i/[K]_o = [H]_i/[H]_o.$$

However, careful determination of the hydrogen ions in muscle fibres has shown that their concentration in the sarcoplasm is about the same as in the surrounding medium (Fenn, 1936, 1940; Conway, 1957). Investigation of this problem by means of the microelectrode technique has also shown that the distribution of hydrogen ions does not obey the Donnan principle (Caldwell, 1954, 1958; Hill, 1955; Sorokina, 1959, 1961; Kostyuk and Sorokina, 1961). Some time ago Netter (Netter, 1928, see also Mond and Netter, 1930, 1932; Höber, 1945) suggested the idea that cations which penetrate a cell are distributed between the medium and the cell in accordance with Donnan membrane equilibrium. However Fenn and Cobb (1934/5) in experiments with isolated frog muscles were unable to confirm Netter's ideas. They found that the potassium concentration in the muscles depends on its concentration in the medium and on the pH of the latter, as can be seen from Fig. 90, but that the deviations from Netter's hypothesis were so large that it was necessary to assume that hydrogen ions are completely incapable of penetrating muscle fibres if the experimental data were to be reconciled with Donnan equilibrium. Clearly it is necessary in this case too to postulate the existence in the membrane of a special pump to pump the hydrogen ions out of the cells.

Further, it has now been convincingly shown that the concentration of free (ionised) calcium in muscle and nerve fibres is many times lower than in the surrounding medium (Keynes and Lewis, 1956; Hodgkin and Keynes, 1957; Dranitskaya, 1960). On the other hand, it is known that calcium ions, just like ions of the other alkaline earth metals, penetrate muscle and nerve fibres (Harris, 1955, 1957a; Flückiger and Keynes, 1955; Bianchi and Shanes, 1959; Shanes and Bianchi, 1959; Mullins, 1959; Frater *et al.*, 1959; Cosmos and Harris, 1961; Dranitskaya, 1960), and that the rate of penetration is significantly increased upon stimulation of the muscles and nerves (Flückiger and Keynes, 1955; Hodgkin and Keynes, 1957; Bianchi and Shanes, 1959, 1960; Shanes, 1960; Winegrad, 1960, 1961; Dranitskaya, 1960). Consequently, according to the membrane theory there should also be a membrane pump capable of continuously carrying calcium ions out of cells.

The ideas of Hodgkin and his colleagues about the functioning of the sodium pump and the connection between the expulsion of sodium from a cell and the entry into it of an equivalent amount of potassium also encounter serious difficulties. Thus, the carriage of sodium out of a cell should cease when there is no sodium and potassium in the medium or when active transport is poisoned; this is not in fact observed (Ussing, 1949; Glynn,

1956; Swan and Keynes, 1956). Radioactive sodium is released from the cuttlefish axon into a pure sucrose solution free of potassium and sodium in the presence of dinitrophenol (Hodgkin and Keynes, 1955a).

According to the sodium pump hypothesis, a strict correlation should be observed between the potassium and sodium concentrations in cells and also between the rates of exchange of these ions for the same ions of the medium. This is also not observed (Shaw and Simon, 1955a, b; Simon *et al.*, 1957; Scott and Hayward, 1954; Elsbach and Schwartz, 1959; Maizels and Remington, 1959a; Steinbach, 1961), though there is complete correlation between the concentrations in the cells of sodium and chloride ions (Simon *et al.*, 1957).

Ling (1952, 1955), criticising the Boyle-Conway theory and the sodium pump hypothesis, adduces facts which indicate that some anions which "do not penetrate" the cell membrane, as certain proteins, organic phosphorus compounds and so on, can in fact, in certain conditions, easily pass through the protoplasmic membrane.

In fact, certain proteins—enzymes can pass out of intact muscle fibres of warm-blooded animals (Zierler *et al.*, 1953; Zierler, 1956, 1957, 1958). It has been shown that frog muscle fibres are permeable for haemoglobin (Meshcherskaya, 1931), creatine, carnosine, nucleotides, and many enzymes (Braun, 1958, 1960; Braun, Nemchinskaya, Nesvetayeva and Sokolova, 1959; Nemchinskaya, 1959). Intact yeast cells are permeable for invertase (Oparin and Yurkevich, 1949; Yurkevich, 1954). It has also been established that haemoglobin, lysozyme and ribonuclease pass into the cytoplasm of barley root cells (McLaren *et al.*, 1960). Vitamin B<sub>12</sub> finds no difficulty in passing into the cells of rat liver slices (Latner *et al.*, 1959). Deoxyribonucleic acid is able to penetrate bacteria (Fox, 1957 and others). Brachet (1957) assembled a large amount of work which shows that many cells are permeable for ribonuclease (Brachet, 1955, 1956) and basic proteins with a molecular weight of up to 10,000 (Ficher and Wagner, 1954 and others).

Carnosine, which is concentrated in cells like creatinephosphate and hexose-monophosphate, easily passes into muscle fibres (Eggerton and Eggerton, 1933). It has been established also that the anion of glutamic acid passes into cells and can accumulate in them in concentrations exceeding that in the medium (Terner, Eggerton and Krebs, 1950).

Ling (1952, 1955) produces a most interesting fact which contradicts the sodium pump hypothesis. Isolated frog muscle that has been poisoned with iodoacetate in an atmosphere of pure nitrogen at 0° preserves its potassium concentration virtually unchanged for 5 hr. There is also very little change in the energy sources (creatinephosphate, ATP) for the sodium pump, if it in fact exists. At the same time there is a considerable flux of sodium ions into the muscle fibres and in the opposite direction. These data cannot be explained from the standpoint of the sodium pump.

Other experimental data will be produced in the following sections of this

chapter which cannot be fitted into the framework of either the classical or modernised membrane theories.

### *2. The State of Mineral Substances in the Protoplasm according to the Sorptional Theory of Cell Permeability*

In the literature on the permeability of cells the suggestion has repeatedly been made that the mineral substances of which the protoplasm is composed are partially dissolved there and are in diffusion equilibrium with the medium, while they are also partially bound with the colloids of the protoplasm, principally with the proteinaceous bodies.

As we shall see below, there is a wealth of factual data and indirect arguments in support of such a mechanism of the distribution of mineral substances between cell and medium.

*Anion deficit.* All workers who have studied the electrolytic composition of plant and animal cells have always found a considerable anion deficit—the cations exceeding the anions in number. Thus, according to Kramer and Tisdall (1922), in human erythrocytes 17 per cent of the total amount of cations (basically potassium, of which there is 428 mg per cent in the cells) is

TABLE 87. THE SALT COMPOSITION OF UNFERTILISED SEA URCHIN EGGS  
(from Page, 1927)

Ion	mg per million eggs	mm	mequiv
Calcium	1.90	0.047	0.094
Magnesium	4.48	0.182	0.364
Sodium	1.301	0.056	0.056
Potassium	2.445	0.063	0.063
Iron	0.030	0.0005	0.0015
Total cations	—	0.348	0.5785
Sulphate	0.00046	0.00004	0.00008
Chloride	0.1864	0.0053	0.0053
Phosphorus (total)	0.9064	0.0291	0.0873
Nitrate	traces	—	—
Total anions	—	0.034	0.0927
Anion deficit	—	—	0.4858

not compensated by mineral acid radicals. The authors suppose that the excess cations are in combination with proteins. Page (1927) found that the molar cation and anion concentration in sea urchin eggs is higher than in the external medium (in sea water), and that there are almost six times as many cations as anions. These interesting results of Page's are set out in Table 87. They show that at least part of the cations are bound with the cell colloids.

Hill and Kupalov (1930) found in the case of frog muscles an anion deficit equal to about 0·058 N, which, in their opinion, is covered by the proteins in the muscle forming ionised proteinate.

According to Fenn's calculations (Fenn, 1936, 1938a) the anion deficit in frog muscles reaches about 47 mequiv. According to Fenn, Cobb, Hegnauer and Marsh (1934/5), there are about twice as many cations in frog sciatic nerve as anions (13·51 mequiv per 100 g nerve of cations to 7·93 mequiv of anions). A considerable amount of these excess cations, at least half, so the authors think, should be bound with the proteins of the protoplasm. According to the analyses of Bear and Schmidt (1939), the axoplasma of the giant nerve fibre of the squid contains 0·5 N excess cations, which are not covered by the presence of chloride in the nerve and cannot be compensated by  $\text{HCO}_3^-$ ,  $\text{OH}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{HPO}_4^{2-}$ . This anion deficit evidently replaces the axoplasm proteins; otherwise, as the authors think, the axoplasma should contain some unknown anions in large amounts.

According to the calculations of Hodgkin (1951) the axoplasma of cephalopod molluscs contains an excess of cations reaching 300 mequiv. per kg of water. This author supposes that part of this excess of cations can be compensated by certain free amino acids, while for the rest some unknown anions must be found. It is noteworthy that there was later found in the axoplasma of cephalopods a high concentration (270 mm/kg) of isethionate, which to a considerable extent makes good this anion deficit (Koechlin, 1955).

Epshteyn (1947) found in rat brain tissue 204 mequiv of bases per 1 kg of fresh tissue, of which only 29 mequiv was in combination with known inorganic acids (chlorides, inorganic phosphates, bicarbonates), while 175 mequiv of these bases were in combination with organic substances (lipids, proteins, amino acids, etc.).

Thus, the anion deficit in the protoplasm gives grounds for supposing that there is present in the cell a considerable amount of cations, especially potassium, which is bound with the protoplasm colloids. However, these data as yet tell us nothing about the state of the mineral cations and anions of that part which is found in cells in equivalent quantities, because part of these anions can also go into high molecular weight compounds and be in the bound state.

*Bound and dissolved mineral substances in the protoplasm.* Many workers have attempted by chemical and physio-chemical methods to establish the amount of potassium ions bound with the protoplasm proteins, because this cation is in the majority of cases present in greater amounts than all other cations.

In a series of papers on their experiments with toad, rabbit and dog muscles, Neuschlosz *et al.* (1923–6) describe their findings that part of the potassium in muscles is in the bound state and does not pass into a pure sodium chloride solution if the pulp of the muscles is placed in it. However, the methodological aspects of Neuschlosz's work cannot be considered satisfactory. Sub-

sequently a number of authors also published criticisms of this author's theoretical set-up. Though using the same technique, they were unable to obtain confirmation of these data in their experiments (Simon and Szelöczey, 1928; Gallison, 1931, and others). Steinbach (1950), experimenting with frog muscle homogenates, found that the precipitated fraction of the homogenate contains a small excess of sodium over potassium. In precipitated material potassium and sodium are replaced by ammonium, calcium and magnesium, potassium being replaced more easily than sodium. It was found that there is in the muscle about 2-3 per cent of sodium which is firmly bound and not replaced by other ions. Harris and Steinbach (1956) showed that this part of the sodium is bound with the connective tissue of the muscles. The precipitated ghosts of the erythrocytes show no selective ability to bind potassium or sodium (Hisashi, 1959); likewise, no such property is found in solutions of a number of nucleotides (Tosteson, 1957). According to Fenn (1957), glycerinised muscles do not bind potassium more than sodium; on the contrary, a small amount of firmly bound sodium is found in these muscles.

In many summaries in which the question is discussed whether proteins are able to bind the ions of the alkaline metals it is indicated that pure proteins in solution are not capable of selective binding of potassium or sodium (Klotz, 1952, 1953; Carr, 1955; Ling, 1952; Robinson, 1960 etc.). Of course, it does not follow from these data that the protoplasm too does not have this property.

The results obtained by Ungar and his colleagues are of great interest. These workers extracted proteins from rat brains and determined the bound (unionised) sodium and potassium in these extracts by measuring the rate of their diffusion through cellophane and by measuring the activity of glass electrodes sensitive to potassium or sodium. It was found that a certain amount of potassium, more than of sodium, was in these extracts in the bound state. On stimulation of the brain through the sciatic nerve the picture changes: in the extracts from excited brain there is more bound sodium found than potassium. At the same time there is a strong increase in the concentration of free SH-groups in the proteins of the tissue (Ungar, Aschheim, Psychoyos and Romano, 1957; Ungar and Romano, 1958, 1959; Ungar, 1959; Ungar and Kadis, 1959; Ungar, 1961).

Ernst and Schaffer (1928), Ernst and Takács (1931), Ernst and Fricker (1934a, b) and Ernst (1953, 1958, 1961) produced a large amount of facts obtained by other methods which show convincingly that part of the potassium in muscle is bound.

Ernst and Fricker (1934a) froze frog gastrocnemius muscles and in the frozen state ground them in a mortar; then from this "muscle snow" they made acetone extracts of potassium. It is supposed that if the potassium in the muscle is bound with the proteins, then it cannot go into solution upon precipitation of the proteins with acetone, because acetone contains no

anions. It was actually found that  $\frac{1}{5}$  to  $\frac{1}{3}$  of the potassium goes into the filtrate from the snow of resting muscles.

The data of Ernst and Fricker were completely confirmed by Reginster (1937), using a similar method. He found that resting frog gastrocnemius muscles contain approximately four times as much bound potassium as diffusible.

In connection with the question of the forms in which mineral substances exist in the protoplasm, great interest attaches to the work of Kometiani and his colleagues (Kometiani, Dolidze and Klein, 1944; Kometiani, Klein and Dolidze, 1946; Kometiani, 1947, 1948a, b). They showed that more than 70 per cent of the intracellular potassium is in the bound state.

Kometiani and his colleagues (1946) determined the amount of free potassium in muscle from the difference of the total potassium and the potassium passing into the precipitate in an alcoholic extract of the muscles. Simultaneously the authors determined the amount of chloride in the muscles, using this as a measure of the magnitude of the extracellular spaces on the assumption that there is no chloride in the muscle fibres. The results are given in Table 88.

It follows from these data that rectus abdominus muscle contains 76 per cent bound potassium, sartorius muscle 84 per cent and gastrocnemius muscle 85 per cent.

TABLE 88. THE POTASSIUM AND CHLORIDE CONTENT OF VARIOUS FROG MUSCLES  
(in  $\mu\text{M}$  per gram of tissue or intracellular water; arithmetic mean of 18 analyses)

(from Kometiani, Klein and Dolidze, 1946)

Muscle	Dry matter %	Chloride $\mu\text{M}/\text{g}$	Extra-cellular space %	Potassium per gram of tissue $\mu\text{M}$		Potassium per gram of intracellular water, $\mu\text{M}$	
				total	bound	total	bound
Rectus abdominus	18.8	15.7	23.0	71.0	53.9	112.6	85.9
Sartorius	19.5	12.2	17.7	74.2	61.7	111.5	93.2
Gastrocnemius	20.7	8.4	12.6	77.0	65.8	110.7	95.1

Kometiani (1948a, b) also showed that potassium and phosphate can bind with soluble muscle proteins (myosin, myoalbumin and globulin-x). The binding of salts was determined by various methods: from the change in the electrical conductivity of the phosphate buffer on the introduction into it of the proteins, by determination of the change of the membrane potential and by determining the potassium in the protein precipitate after treatment with acetone. It was noted that with increasing pH below 6.8-6.5 there is increased binding of potassium by myosin and myoalbumin, while at pH higher than 6.8, on the other hand, there is a decrease (Fig. 91).

Ernst's conclusions about the state of potassium in muscle later found confirmation in the laboratory of Szent-Györgyi (1948). The latter attaches great significance to the binding and liberation of potassium in the course of the conversions of actomyosin complexes, which cause muscle contraction. Szent-Györgyi's colleagues established the reversible binding by myosin solution of the ions of potassium, calcium, magnesium etc., and also ATP.

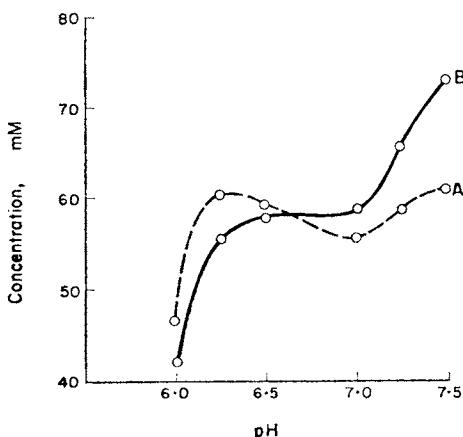


FIG. 91. The binding of potassium ions by dissolved myosin at various values of pH (A) in the presence and (B) in the absence of acetylcholine (from Kometiani, 1948a).

The myosin crystals which fall out of dilute potassium chloride solution and separate from the surrounding liquid form on treatment with refrigerated alcohol a denatured myosin powder which contains a considerable amount of potassium and is free from chloride. Thus, myosin binds potassium and does not bind chloride. Myosin preliminarily denatured does not bind potassium. When myosin is denatured with alcohol in the presence of anions the potassium splits off from the myosin and goes into solution. The affinity of myosin for potassium falls sharply with time. If myosin solution is kept for 48 hr at 0°, the binding of potassium is reduced to about half.

Magnesium has a greater affinity for myosin than potassium. If calcium and magnesium are simultaneously present in the solution, the calcium is adsorbed preferentially before the magnesium and the potassium. The absorption of potassium, calcium and magnesium by myosin is a reversible process. If the salt concentration in the medium is lowered, it becomes easier to split off the potassium from the myosin, but harder to split off the magnesium and calcium. Myosin also forms a reversible complex with ATP. ATP on combining with myosin facilitates the absorption by myosin of cations as well. If ATP is bound by myosin in the presence of potassium, calcium and magnesium, then on precipitation of the myosin with alcohol, it

is potassium that preferentially goes into the precipitate. Actomyosin binds potassium in the same quantity as myosin and calcium and magnesium twice as much.

According to Mullins (1942a), pieces of myosin gel, prepared from fresh rabbit muscle myosin and placed in an equimolar solution of potassium and sodium chloride (total concentration 0·2 M) contained 3·7 times more potassium than the medium and only 1·1 times as much sodium. The effectiveness of the absorption of potassium by myosin is raised by increasing the pH to 8, when the protein is strongly ionised. If the temperature is raised above 37°, the myosin is denatured, after which it loses its power of accumulating potassium. The preferential accumulation of potassium rather than sodium cannot be explained by the difference in their rate of diffusion into the myosin gel, because these data were obtained in conditions of diffusion equilibrium: identical results were obtained after 1 and after 24 hr. In the author's opinion, this property of myosin, that it accumulates potassium and not sodium, makes it possible to explain the "anomalous distribution of potassium between muscle and plasma" (p. 62). However, according to Szent-Györgyi and his colleagues (Szent-Györgyi, 1948, 1957) myosin solution has no preferential "affinity" for potassium over sodium. The same was found by Lewis and Saroff (1957).

Stone and Shapiro (1948) established by ultrafiltration that homogenates of rat muscle and brain contain from 25 to 33 per cent of bound potassium. The authors supposed that the potassium is bound preferentially with the lipids.

Tarusov and Burlakova (1939) in experiments with rabbit erythrocytes found that erythrocytes haemolysed by hypotonic solution do not give off potassium into the medium and preserve completely their polarised properties. On haemolysis with saponin the potassium comes out of the cell with the haemoglobin and the polarisational properties of these cells fall simultaneously. On the basis of their experiments the authors came to the conclusion that the obtained "results exclude the possibility that the potassium in the erythrocytes is in a completely free state; it is unquestionably bound with protein" (p. 394).

Solomon, Hald and Peters (1940) studied the state of the inorganic components of human erythrocytes by means of ultrafiltration through cellophane. Ultrafiltration of blood haemolysed by refrigeration (normal blood, and also blood preserved cold and at 37°) showed that a considerable amount of potassium, calcium and phosphorus does not go into the ultrafiltrate. The potassium, in their opinion, is bound with protein and so is unable to diffuse; the sodium is also partially bound with the organic phosphates and is not diffusible.

According to the calculations of Rozenberg (1955), there are in erythrocytes considerable quantities of chloride and bromide, iodide, etc. passing into these cells which should be adsorptionally bound by the colloids of the stroma of the blood corpuscles.

In confirmation of this view results can be adduced from many workers which show that proteins in solution are capable of binding orthophosphate and other mineral anions (Soreni and Chepinoga, 1946; Soreni, 1947; Kometiani, 1948a, b; Velick, 1949; Klotz and Urquhart, 1949; Cannan and Levy, 1950; Horowitz, 1959; Klotz, 1953; Carr, 1955 etc.). Mineral anions differ in their capacity for being bound by protein solutions. Thus, in order of increasing ability to be bound by serum albumin, anions form the following series (Carr, 1955):



The ability of dissolved proteins to bind the cations of the alkaline earth metals has been investigated by many writers (Szent-Györgyi, 1948; Klotz, 1952, 1953; Carr, 1955). Calcium and magnesium ions are bound in large quantities by myosin (see Szent-Györgyi, 1948) and also by serum albumin and pepsin (Carr, 1955). According to this author, the affinity for calcium and magnesium falls off in the following series of proteins: pepsin, serum albumin, lysozyme, egg albumin, serum globulin with haemoglobin a poor last.

In homogenates prepared from muscles (Weise, 1934; Berwick, 1951; Weimar, 1953) and eggs of sea urchins (Gross, 1954) a large amount of bound calcium is found. The amount is reduced by the action of elevated temperature, narcotics and other agents.

Thus, the ability of solutions of pure proteins and homogenates of tissues to bind mineral cations and anions indicates that it is in principle possible for there to be in cells a certain amount of "bound" mineral substances.

Many workers analysing the data on the absorption and release of potassium, sodium, chloride and other ions by frog muscles in media containing various concentrations of these ions, have concluded that the distribution of potassium between muscles and medium can be explained on the assumption that a considerable part of the muscle potassium is bound (Steinbach, 1940a, b, 1944, 1947; Dubuisson, 1942; Nasonov and Aleksandrov, 1943; Wilde, 1945; Ernst, 1928, 1958; Troshin, 1953a; Nasonov, 1959 etc.).

Krogh, Lindberg and Schmidt-Nielsen (1944) and Krogh (1946), discussing the question of the existence of non-diffusible potassium in the protoplasm, came to the conclusion that muscle fibres should contain (in the unionised state) at least 60 per cent of the potassium that cannot be replaced by ionised potassium from the blood plasma. In their opinion, this part of the potassium is in chemical combination with some organic acids of unknown nature; they should be of small molecular weight, numerous, and their compounds with potassium should be unionised and incapable of diffusing through the cell membrane. Thus, so these authors suppose, osmotic equilibrium is achieved between the cells and the plasma and the anion deficit is made good.

To determine the amount of free and bound potassium a group of workers have applied labelled atom techniques. Potassium chloride with an admixture of radioactive potassium is introduced into the blood of the animal and the change in its specific radioactivity with time is determined. If the potassium of the plasma and that of the organs do not differ in their

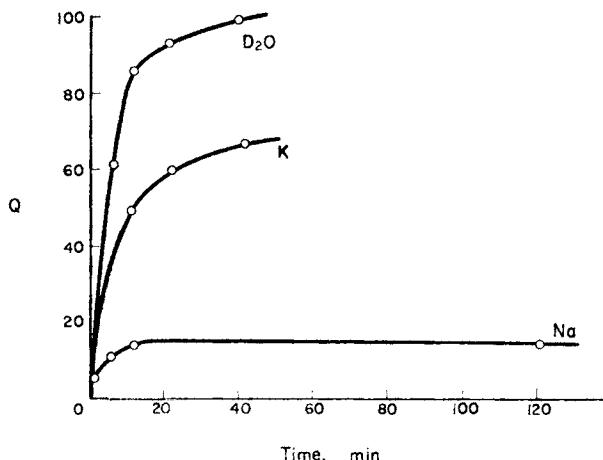


FIG. 92. The change with time of the distribution coefficients ( $Q$ ) of radioactive potassium, sodium and heavy water between rabbit skeletal muscles and blood plasma (from Hahn and Hevesy, 1941a).

specific radioactivity, this means that the whole of the cell potassium has been replaced in the course of the experiment with blood plasma potassium. By this method Hevesy and Hahn (1941) and Hahn and Hevesy (1941a, b, 1942) found that up to 60 per cent of the intracellular potassium is bound.

In Fig. 92, which is taken from Hahn and Hevesy (1941a), the change with time is shown of the coefficient of distribution of the radioactive potassium and sodium and also of heavy water between rabbit skeletal muscles and plasma. The potassium and sodium concentrations were calculated per 100 g of muscles and plasma water. It can be seen in the figure that in 40 min the distribution coefficient of heavy water reaches 100 per cent, i.e., all the muscle water is exchanged for plasma water. In the first 10 min the sodium distribution coefficient reaches 15 per cent and then remains on its former level for 2 hr. If we remember that the sodium concentration in the muscles is about one fifth of that in the plasma, it follows that almost all the sodium of the muscles (about 80 per cent) has been exchanged for plasma sodium already in the first 10 min. The potassium distribution coefficient reaches 65 per cent in 40 min and thereafter increases very slowly. This means that the concentration of free potassium in the muscle, which is capable of easy

exchange for protoplasma potassium, is considerably less than its concentration in the protoplasma.

In another paper Hahn and Hevesy (1941 b) showed that rabbit and rat muscles, and also rat brain and liver, exchange their intracellular potassium for potassium of the plasma only to 30–40 per cent in all over a lengthy period (in 24, 48 and 64 hr). On the basis of these data the authors came to the conclusion that about  $\frac{2}{3}$  of the intracellular potassium is bound.

According to Mullins, Fenn, Noonan and Haege (1941) only 20 per cent of the potassium of frog erythrocytes is exchanged for  $^{42}\text{K}^+$  of the plasma (Fig. 93). Approximately the same results are obtained in experiments with rats and dogs. In bull, rabbit and guinea pig erythrocytes about 40 per cent of the potassium is exchanged, while in cat erythrocytes almost all the potassium is exchanged in the first 5 hr for potassium of the plasma.

Hevesy and Hahn (1941), and also Hahn and Hevesy (1941 b, 1942) found that rabbit, dog and rat erythrocytes have 30–35 per cent in all of their total intracellular potassium exchanging for potassium of the plasma. Mineral phosphorus behaves similarly. In contrast to potassium, all the chloride and almost all the sodium in rabbit, cat, dog and rat erythrocytes is present in

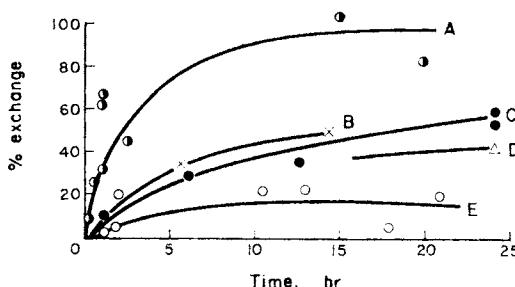


FIG. 93. The exchange of potassium of erythrocytes for potassium of plasma (from Mullins, Fenn, Noonan and Haege, 1941).

A—cat; B—guinea pig; C—rabbit; D—bull; E—frog.

the free state and is easily exchanged for the same ions of the plasma. This can be seen well in Fig. 94. In this figure the distribution coefficients, which are 0.56 for chloride and phosphate and 0.20 for sodium, show that all the free ions of chloride, sodium and inorganic phosphorus of the erythrocytes are exchanged for ions of chloride, sodium and phosphoric acid of the plasma.

Fenn and his colleagues (Fenn, Noonan, Mullins and Haege, 1941; Mullins, Fenn, Noonan and Haege, 1941; Noonan, Fenn and Haege, 1941 a) used the method of labelled atoms to study the state of the potassium in the cells of various organs of vertebrates but obtained contradictory data. According to their results, only erythrocytes contain a significant amount of bound potassium (see Fig. 95).

In another paper, the same writers (Fenn, Noonan, Mullins and Haege, 1941) determined the amount of changing intracellular potassium in the body from the change with time of the specific activity of the potassium of the urine or plasma. This method revealed that in 5-10 hr only 50 per cent of the intracellular potassium of the human and rabbit body are exchanged, the remainder failing to do so in the course of 24 hr and more. Similar results were obtained by Hevesy (1942).

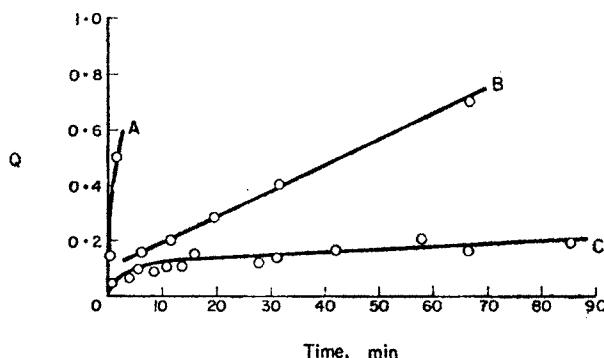


FIG. 94. The exchange of chloride (A) and phosphorus (B) between rabbit erythrocytes and plasma and the exchange of sodium (C) between dog erythrocytes and plasma (from Hahn and Hevesy, 1942).

The ordinates give the ratios ( $Q$ ) of the concentrations of radioactive chloride, phosphorus and sodium in the erythrocytes and in the plasma.

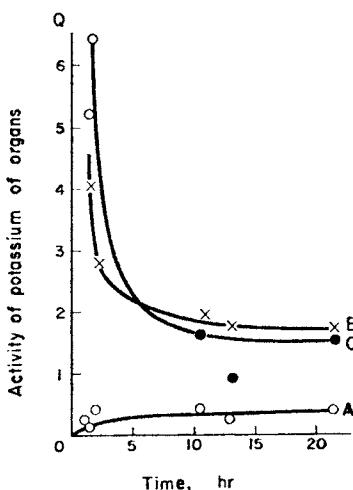


FIG. 95. The exchange of potassium of the erythrocytes (A) and heart (B) of frogs for radioactive potassium of the plasma (C). The relative specific activity of potassium, equal to 1.5, corresponds to complete exchange of cell potassium for plasma potassium (from Fenn, Noonan, Mullins and Haege, 1941).

It should be noted that the data cited cannot reflect exactly the amount of free potassium in the cell, because in the course of the experiment part of the bound potassium can split off and a certain amount of the free potassium (including the radioactive) can become bound.

Further, the results of numerous modern studies made on individual cells or isolated tissues indicate that all the cell potassium is exchanged for labelled potassium of the medium (Harris and Burn, 1949; Sheppard and Martin, 1949, 1950; Keynes and Lewis, 1951b; Harris and McLennan, 1953; Troshin, 1960 etc.). It is, however, noted in many papers that one part of the cell potassium exchanges rapidly and another slowly. This means that the potassium in the protoplasm does not form a single homogeneous fraction. These results were obtained by Harris and his colleagues in studying the rate of displacement of cell potassium by radioactive potassium of the medium in experiments with isolated frog muscles (Harris, 1952, 1953), with cells of the sympathetic ganglia and nerves of warm-blooded animals (Harris and McLennan, 1953; McLennan, 1953a, b, 1956; McLennan and Harris, 1954), with frog sciatic nerve (Harris and Nicholls, 1956) and rat muscles (McLennan, 1955, 1956). They were able to establish the existence in cells of at least three potassium fractions, of which one (up to 50 per cent of the cell potassium) is exchanged at a very low rate or not at all. It was supposed that this potassium is bound with the protein and is unionised (Harris, 1953).

Later, however, another explanation was given for this phenomenon. According to Harris, there are two regions in the cell: an outer region (near the cell surface, which may include the reticulum too), in which the exchanging ions are adsorbed and where the principal concentration of cell sodium forms, and an inner region of the cell, in which potassium ions are accumulated. The process of the exchange of potassium ions of the cell for the same (or other) ions of the medium is determined by the exchange of adsorbed ions in the outer region of the cell with external ions, by the exchange of ions of the outer region with ions of the inner region and by the diffusion of ions in the inner region (the potassium region). The process of the exchange of ions in the external region of the cell, which has ion-exchange properties, takes place slowly (Harris, 1957, 1958, 1960; Harris and Prankerd, 1957; Edwards and Harris, 1957; Edwards, Harris and Nishie, 1957; Harris and Sjodin, 1959, 1961; Cosmos and Harris, 1961; Bolingbroke, Harris and Sjodin, 1961).

It is probable that the exchange of cell potassium for potassium of the medium is a more complex process than a first order one; it includes several exponential components and not one, as should be the case if the barrier for ion exchange were only the cell membrane (Troshin, 1960, 1961; Simon, 1961 etc.).

In our laboratory (Troshin and Pisareva, 1958; Troshin, 1960) a study has been made of the kinetics of the potassium exchange of frog sartorius muscle for labelled potassium of Ringer's solution. The extent of the replace-

ment of muscle potassium by medium potassium in a fixed time interval was determined side by side with the total concentration of potassium in the muscles (by means of a flame spectrophotometer). The results are shown in Fig. 96. They show that the exchange of muscle potassium takes place rapidly at first and then slowly.

Analysis of the curve of the displacement of muscle potassium by labelled potassium of the medium shows that it is made up of at least three exponential components and may be described sufficiently accurately by the following equation:

$$K_m = Ae^{-at} + Be^{-bt} + Ce^{-ct},$$

where  $K_m$  is the undisplaced potassium in the muscles at a time  $t$ ,  $A$ ,  $B$  and  $C$  the initial concentrations of the three fractions of muscle potassium, and  $a$ ,  $b$  and  $c$  the time constants of their exchange. In Table 89 we show the con-

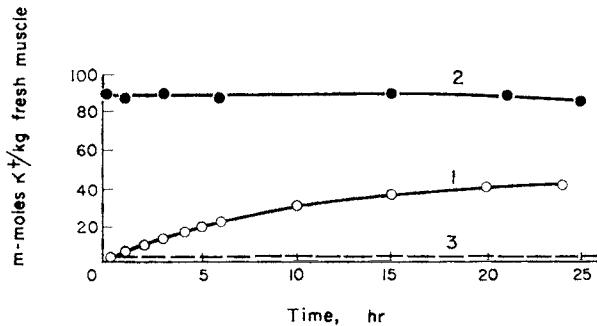


FIG. 96. The displacement of potassium of muscle by labelled potassium of Ringer's solution.

1—the concentration of displaced potassium of the muscles by labelled potassium of the medium; 2—the concentration of potassium in the muscles chemically determined; 3—the potassium concentration in the intercellular spaces. Temperatures 18–20°.

TABLE 89. POTASSIUM EXCHANGE CONSTANTS FOR FROG SARTORIUS MUSCLE IN RINGER'S SOLUTION. TEMPERATURE—18–20° (Troshin, 1960a)

Potassium fraction of muscle	Initial concentration of undisplaced potassium in muscles (at $t = 0$ )		Time constant (hr $^{-1}$ )
	%	mequiv %	
<i>A</i> ("fast")	3.1	0.2	$a = 4.401$
<i>B</i> ("medium")	15.6	1.3	$b = 0.243$
<i>C</i> ("slow")	81.3	7.2	$c = 0.020$
Total potassium	100	8.7	—

stants of this equation. It can be seen that the "quickest" fraction of labelled potassium *A* is really very quickly exchanged by comparison with the *B*, and especially the *C*, fraction. The *A* potassium fraction is concentrated not only in the intercellular spaces; considerably more than half of this fraction belongs to the muscle fibres. Thus, the potassium of muscle fibres is not a homogeneous fraction and cannot mix entirely without hindrance with the potassium of the medium that penetrates the fibre.

Confirmation of the accuracy of this proposal can be found in the convincing data of Harris and Steinbach (1956). They incubated frog skeletal muscles for a period in a solution containing radioactive sodium or potassium ions and then excreted these elements from the muscles by treatment with successive lots of distilled water or sucrose solution. It turned out that the specific activity of the sodium and potassium extracted from the muscle in the first lot was considerably higher than in the subsequent lots and the rest of the sodium and potassium in the muscles. Similar results were also obtained in Ernst's laboratory (Ernst and Hajnal, 1959) in experiments with muscles for potassium.

The experimental data of Sorokina (1961) on the amount of potassium and sodium ions passing from muscles into solution in acetone extracts and also on the distribution of radioactive potassium, sodium and chloride between muscles and Ringer's solution showed that the easily exchanging part in frog sartorius muscles is 29 per cent in all for potassium, 82 per cent for sodium and 100 per cent in the case of chloride ions, the exchange being completed in as little as 40 min. The author supposed that the slowly changing part of the muscle potassium and sodium is adsorbed by the protein and other large anions of the muscles (see Sorokina, 1961; Kostyuk, Sorokina and Shapovalov, 1959).

In experiments on other subjects many authors have also found that a considerable part of the intracellular potassium is not exchanged at all or is exchanged only at a very slow rate for radioactive potassium of the medium. One may cite experiments with micro-organisms (Cowie, Roberts and Roberts, 1949; Eddy and Hinshelwood, 1950), with the earth amoeba *Acanthamoeba sp.* and their cysts (Klein, 1959), with unfertilised eggs of marine animals (Chambers, White, Jeung and Brooks, 1948), with erythrocytes (Solomon and Gold, 1955; Tosteson and Robertson, 1956 etc.), with slices of rabbit kidney cortex (Mudge, 1952), with single giant squid fibres (Rothenberg, 1948, 1950) and with isolated hearts (Humphrey and Johnson, 1960; Klein, 1960). Menozzi *et al.* (1959) showed that rubidium ions, which penetrate the muscle fibres of rat diaphragms expel a corresponding amount of the potassium adsorbed in the sarcoplasm and bind in the same places where the potassium was adsorbed.

With regard to the state of the sodium in cells the researches of many authors have shown that a small part of this is present in the protoplasm in a firmly bound form, but that the greater part of this cation is free. The bound

sodium of cells does not emerge into a medium that is free of sodium ions. This has been convincingly demonstrated in experiments with muscles (Fenn, Cobb and Marsh, 1934/5; Tobias, 1950; Carey and Conway, 1954; Conway and Carey, 1955; Harris and Steinbach, 1956). A number of writers have also shown that the bound sodium of muscle fibres, erythrocytes and egg cells does not exchange at all or exchanges only very slowly for radioactive sodium of the medium (Cohn and Cohn, 1939; Hahn and Hevesy, 1941 a; Manery and Bale, 1941; Abelson and Duryee, 1949; Solomon, 1952a; Gold and Solomon,

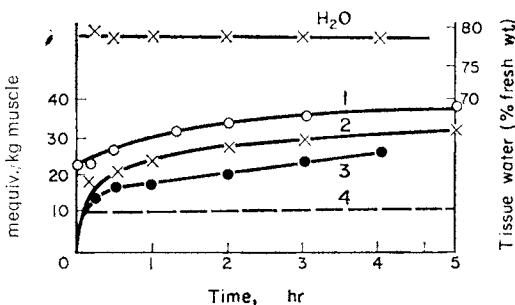


FIG. 97. The displacement of sodium of frog sartorius muscles for radioactive sodium and lithium ions of Ringer's solution.

1—the total amount of sodium in the muscles; 2—the amount of sodium of the muscles displaced for labelled sodium of the medium; 3—the lithium concentration in the muscles; 4—the sodium and lithium concentrations in the intercellular spaces. Temperature 18–20°.

1955; Love and Burch, 1953; Harris and Pranker, 1953; McLennan and Harris, 1954; Carey and Conway, 1954; Conway and Carey, 1955; Solomon and Gold, 1955; Harris and Steinbach, 1956; Troshin, 1957; Troshin and Pisareva, 1958; Klein, 1960 etc.). According to these authors, from 5 to 20 per cent of the cell sodium is bound and so not exchanged.

In Fig. 97 (Troshin, 1957; Troshin, 1961) the change is shown of the sodium concentration in frog sartorius muscles with time of incubation in Ringer's solution and of the displacement of muscle sodium by radioactive sodium of the medium. The curves in this figure show that the basic mass of the muscle sodium is replaced in less than 1 hr by the labelled sodium of the Ringer's solution, while only about 1 per cent of the sodium is replaced slowly. This amount of sodium of the muscles is not replaced by lithium ions, if these are present in the medium in place of sodium. As in the case of the exchange of muscle potassium, the curve of the sodium exchange can be analysed into a minimum of three exponential components. The relevant data from this analysis are shown in Table 90. The most rapidly exchanging sodium fraction makes up about 30 per cent of the total muscle sodium. It is probable that this sodium is concentrated in the intercellular

spaces of the muscle. In this case the intercellular spaces should make up 9·5 per cent of the total muscle volume.

Some workers explain the divergence of the process of the exchange of cell potassium and sodium for the corresponding ions of the medium from a first order process (from a uni-exponential function) by the fact that the experiments were as a rule conducted on cell populations or several organs. In this case we have to do with cells or fibres which can vary in size, in the

TABLE 90. SODIUM EXCHANGE CONSTANTS FOR FROG SARTORIUS MUSCLES IN RINGER'S SOLUTION. TEMPERATURE -18-20° (Troshin, 1957)

Sodium fraction of muscles	Initial concentration of undisplaced sodium in the muscles* (at $t = 0$ )		Time constant (hr <sup>-1</sup> )
	%	mequiv %	
A ("fast")	30	1·05	$a = 23·8$
B ("medium")	50	1·75	$b = 3·89$
C ("slow")	20	0·70	$c = 0·032$
Total sodium	100	3·5	

\* Before immersion in Ringer's solution containing radioactive sodium the muscles were kept for 2·5 hr in a non-radioactive Ringer's solution.

state of the cell membrane and so on (Sheppard, Martin and Beyl, 1951; Carey and Conway, 1954; Garoutte and Aird, 1956; Conway, 1957; Hodgkin, 1958; Hodgkin and Horowicz, 1959; Maizels and Remington, 1959a, Glynn, 1961 etc.).

The matter was subjected to experimental test in experiments with single isolated fibres of frog semitendinosus muscle by Hodgkin and Horowicz (1959). First the muscle fibres were placed for a short time in Ringer's solution containing radioactive potassium or sodium ions. It was then found that the loss of these ions by the fibre into non-radioactive Ringer's solution obeys an exponential law characteristic of a system with one barrier (the cell membrane) preventing the free mixing of cell ions with the corresponding ions of the medium.

However, the conclusions of Hodgkin and Horowicz can hardly be considered convincing because their experimental conditions prevented their tracing the release of ions from the fibre in the first 3-5 min; they could, therefore, have missed a fast component of the exchange process. Furthermore, it is hardly possible to use the release of labelled potassium or sodium that has earlier been introduced into the cell as a guide to the state of the total potassium or sodium in the cell, because in this case the labelled ion that has freely entered the cell will equally freely move out of the cell in exchange for an external ion, if it has not yet had time during its period in the

fibre to replace a bound cation. And, in fact, recent work by Ling (1961), also done with individual frog muscle fibres using a slightly different technique, has shown that the process of potassium exchange from muscle to medium does not obey any simple exponential law.

A frequently used proof of the fact that all the potassium and sodium in cells is in the free form is data on the measurement of the mobility and diffusion coefficients of these ions in nerve and muscle fibres. Thus, Hodgkin

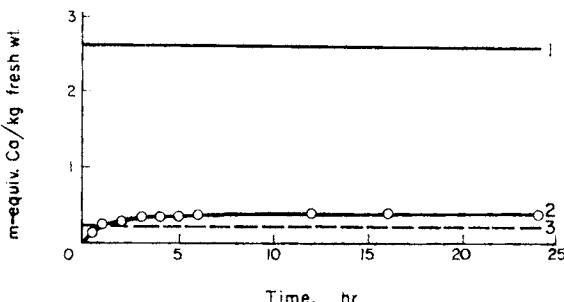


FIG. 98. The displacement of calcium of the muscles for calcium of Ringer's solution (after Dranitskaya, see Troshin, 1961).

1—the total amount of calcium in the muscle; 2—the amount of calcium of the muscle displaced for labelled calcium of the medium; 3—the amount of calcium in the intercellular spaces. Temperature 18–20°.

and Keynes (1953) introduced locally into the giant nerve fibre of cuttlefish radioactive sodium and potassium and then created a small electrical gradient along the length of the fibre. They found that the mobility of these labelled ions in the fibre was the same as in a normal aqueous solution. Similar work with the same result was done by Harris (1954) on frog muscle fibres. The measured diffusion coefficients for sodium and potassium in nerve fibre turned out the same as in an aqueous solution (Hodgkin and Keynes, 1953, 1956; Caldwell and Keynes, 1960).

In all these studies the subject under discussion is the mobility of labelled sodium and potassium in fibres with complete disregard of quantity. It seems strange that the mobility and diffusion coefficients of sodium and potassium in nerve and, especially, in muscle fibres are the same as in water, when we remember the complex picture of their submicroscopic structure. In a recent paper Ling (1961 b) showed that the diffusion coefficient of caesium in frog muscle fibres is many times lower than in an aqueous solution.

A number of authors have used various methods, including radioactive isotopes, to show that a considerable amount of the calcium in muscles (Weise, 1934; Dubuisson, 1942; Berwick, 1951; Weimar, 1953; Harris, 1955; Bianchi and Shanes, 1958, 1959, 1960; Shanes and Bianchi, 1959, 1960; Frater, Simon and Shaw, 1959; Cosmos and Harris, 1961 etc.), in nerve

fibres (Chambers and Kao, 1950, 1952; Keynes and Lewis, 1956; Hodgkin and Keynes, 1957), in erythrocytes (Bolingbroke and Maizels, 1959), in the eggs of sea animals (Mazia, 1937, 1940; Gross, 1954) and in other animal cells (see Heilbrunn, 1956) is in the bound state. According to Harris (1955) the free (ionised) calcium in muscle fibres which can be exchanged for  $^{45}\text{Ca}$  amounts to 10–20 per cent in all of the total calcium; in nerve fibres the amount is even less than this (Keynes and Lewis, 1956; Hodgkin and Keynes, 1957). In our laboratory Dranitskaya (see Troshin, 1961) found that in all about 10 per cent of the calcium of isolated frog muscles is exchanged for radioactive calcium from Ringer's solution. These data are set out in Fig. 98.

As in the case of calcium, a considerable part of the magnesium in the protoplasm is present in the bound form (Dubuisson, 1942; Frater, Simon and Shaw, 1959; May and Barnes, 1960 etc.).

In Mirsky's laboratory (Naoara, Naoara, Mirsky and Allfrey, 1961) experiments were conducted on isolated thymus nuclei in which it was found that the nuclei contain 0·024 per cent calcium and 0·115 per cent magnesium. A considerable part (up to 64 per cent) of the magnesium was bound with nucleic acids (through the phosphate group of these acids) and nucleotides.

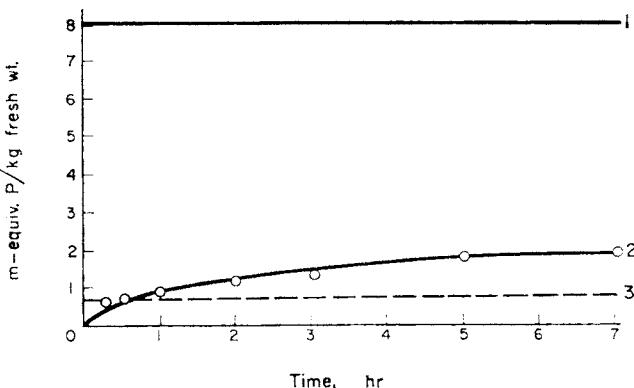


FIG. 99. The displacement of inorganic phosphorus of frog sartorius muscles for radioactive phosphate of Ringer's solution (from the data of Pisareva, 1959).

1—the total concentration of inorganic phosphorus in the muscles; 2—the concentration of displaced phosphate in the muscles; 3—the concentration of inorganic phosphorus in the intercellular spaces. Temperature 2–4°.

It is known that the concentration of so called "inorganic phosphorus" in muscle fibres is many times greater than in blood plasma (see Fenn, 1936; Ling, 1955; Conway, 1957; Panteleyeva, 1953; Pisareva, 1959 etc.), but that in erythrocytes it is about half that in blood plasma (Weir and Hastings, 1939; Hevesy, 1942; Hahn and Hevesy, 1942 etc.). In our laboratory Pisareva (1958, 1959, 1961) showed (see Fig. 99) that of 40 mg per cent inorganic phosphorus only 2 mg per cent is exchanged for radioactive orthophosphate of

Ringer's solution upon inhibition of the incorporation of labelled phosphate into the organic phosphorus compounds of the muscle (temperature 2°, treatment with sodium azide and 2,4- $\alpha$ -dinitrophenol). She supposes that one part of the "inorganic phosphorus" in the cells, which is chemically determinable, is adsorptionally bound with the proteins, while the other part belongs to some labile organic phosphorus compounds which decompose when the phosphorus is extracted from the cells. The possibility of such an origin for "inorganic phosphorus" was earlier indicated by many authors (Kometiani, 1948a, b; Kamen and Spiegelman, 1948; Elliott and Hevesy, 1950; Cannan and Levy, 1950; Chambers and White, 1954; Brooks and Chambers, 1954, etc.). According to Briner, Simon and Shaw (1958) and Simon (1959) the greater part of the inorganic phosphorus in muscles is present in the adsorbed form.

The fact that a considerable part of the cell "inorganic" phosphorus is present in cells in the bound form evidently explains another fact, namely, that the specific radioactivity of this phosphorus, when chemically separated from the cells, remains for a long time lower than the specific radioactivity of the inorganic phosphorus of the medium. This fact emerged from the work of many writers (Hevesy and Aten, 1939; Hahn and Hevesy, 1942; Mullins, 1942b; Furchtgott and Shorr, 1943; Sacks, 1944a, b, 1948; Juni, Kamen, Spiegelman and Wiame, 1947; Kamen and Spiegelman, 1948; Kamen, 1949; Villee *et al.*, 1949; Lindberg, 1950; Mueller and Hastings, 1951; Eichler and Schmeiser, 1951, etc.).

The presence in the protoplasm of bound potassium is also indicated by its very slow emergence from muscles upon perfusion with solutions containing no cations of this element or only a small quantity. Thus, Ernst and Schaffer (1928) showed that frog muscle when perfused for 5–6 hr with Ringer's solution by Trendelenburg's method loses up to 6 per cent in all of its potassium and up to 15 per cent when perfused with potassium-free Ringer's solution.

Further, Ernst and Takács (1931) perfused frog muscles with 8·1 per cent and 7·5 per cent sucrose solutions and found that in a short time the muscles lose all their sodium and chloride, while the potassium and phosphate content changes little or nothing over a long period. On perfusion of muscles with sugar solution at 0–5° for 4 days loss of only 10 per cent of the potassium is observed. When resting muscles are washed with 1 per cent potassium chloride solution they absorb a certain amount of potassium ions, whereas, if excited by electrical current, they release them into the washing fluid. The authors concluded from these results that of 0·160 moles ascribable to potassium and phosphate ions a considerable part is adsorptionally or chemically bound with the muscle proteins and is in a non-diffusible state.

Ernst and Fricker (1934b) produced results showing that the perfusion of frog muscles with normal Ringer's solution for 3–4 days leads to the loss of only 10–15 per cent of the muscle potassium. Because only this amount of

potassium is released from the muscle fibres, and the principal part of this ion is not released from the muscles on washing, one should conclude that it should be bound.

Similar results were obtained by Tron (1952) in a study of the mechanism of the distribution of inorganic ions between the crystalline lens and the intraocular fluid of the eyes of bulls. According to Tron's findings, the crystalline lens contains 57 mg per cent of chloride, 63·6 mg per cent of inorganic phosphorus, 302 mg per cent of potassium and (according to other writers) 61 mg per cent of sodium. In the anterior chamber of the eye there was found to be 437 mg per cent of chloride, 2·8 mg per cent of inorganic phosphorus, 19 mg per cent of potassium and 339 mg per cent of sodium. These ions are present in the same concentrations in the hyaloid membrane. Thus, the distribution of inorganic ions between the crystalline lens and its surrounding fluid is the same as occurs between muscles and blood plasma, between erythrocytes and plasma and so on. Tron placed the isolated crystalline lens in a 4·5 per cent glucose solution for 20 hr at room temperature and found that in this time 96·8 per cent of the chloride, 36·0 per cent of the inorganic phosphorus and 58·3 per cent of the potassium diffused from the lens into the glucose solution. On the basis of these results he concluded that the distribution of inorganic ions between the crystalline lens and the ocular fluids cannot be explained by Donnan membrane equilibrium, and that the potassium and inorganic phosphorus present in the protoplasm of the crystalline lens fibres are there partially in the bound state, whereas the chloride is there in the dissolved state and ionised.

Almost all the sodium and chloride very quickly leaves the muscles and nerve fibres if these are placed in a medium free of these ions (for example, an isotonic sugar solution), whereas potassium ions in such conditions have difficulty in leaving and disappear completely from the cells only upon their death (Overton, 1902a; Urano, 1908; Fahr, 1909; Fenn and Cobb, 1934/5; Fenn, Cobb, Hegnauer and Marsh, 1934/5; Steinbach, 1940a, b, c; Boyle and Conway, 1941). Particularly convincing are results of the kind found in the case of single giant squid fibres (Steinbach, 1941; Steinbach and Spiegelman, 1943, etc.).

From a study of the release of potassium from erythrocytes Ponder (1949, 1950, 1951) came to the conclusion that part of the potassium in cells is in a non-diffusible form.

This proposition is demonstrated pictorially by the curves in Figs. 100 and 101, taken from Steinbach (1940a) and Fenn, Cobb, Hegnauer and Marsh (1934/5). Figure 100 shows the potassium loss by frog muscles into a potassium-free Ringer's solution and Fig. 101 the release of potassium, sodium and chloride ions from frog nerve into a dextrose solution. In Fig. 102, which is taken from one of my papers (Troshin, 1960a), is shown the change in the concentration of potassium in frog sartorius muscles when these are kept in a potassium-free Ringer's solution. The straight line in this figure is the slow

exponential component of the process of potassium loss by the muscle. The initial concentration of this "slow" potassium fraction is 69 mM/kg, or about 80 per cent of the total muscle potassium.

Calcium is seen to behave similarly when tissues are immersed in solutions free of this cation. The evidence is provided by much published material and also by the following results of my own experiments.

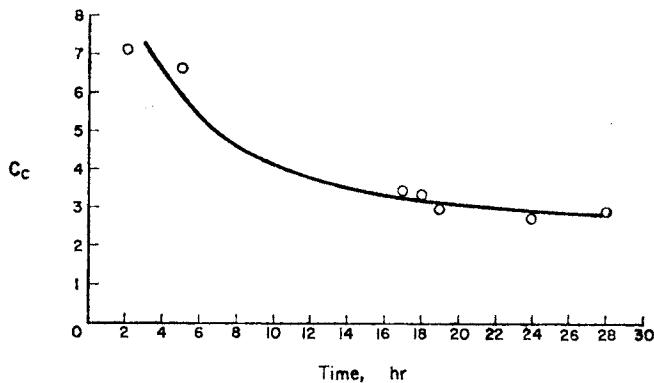


FIG. 100. The loss by frog muscles of potassium with time in Ringer's solution free of potassium (in mequiv per 100 g of fresh weight of tissue) (from Steinbach, 1940a).

Prepared frog gastrocnemius muscles were immersed in Ringer's solution free of calcium ions. The amount of this solution was equal to the weight of muscle. At definite intervals determinations were made of the amount of calcium which had been released from the muscles into the surrounding medium. The experiments were carried out at a temperature of 15–18°. The results are

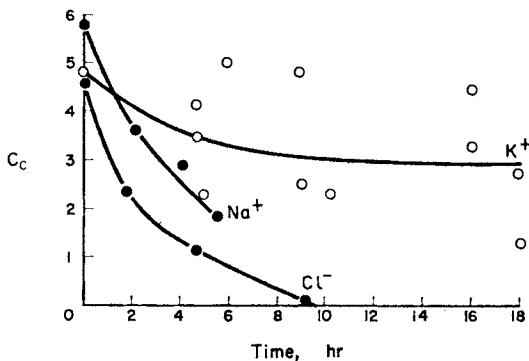


FIG. 101. The reduction in the amount of potassium, sodium and chloride ions in frog nerve immersed in 4.5 per cent dextrose solution (in mequiv per 100 g of nerve) (from Fenn, Cobb, Hegnauer and Marsh, 1934/5).

shown in Fig. 103. The curve A shows the decrease in the calcium in the muscles and the curve B its increase in the medium.

These curves show that the muscles lose in the first 5 hr about 1.25 mg per cent from a total calcium content in the tissue of 11 mg per cent. Further loss of calcium in the muscles takes place very slowly: the additional loss suffered in the next 40 hr is approximately 1.2 mg per cent. After 24 hr in the solution the muscles contain about 8 mg per cent calcium. This quantity is in equilibrium with a 2.5 mg per cent solution of calcium ions in the medium. This ratio of the concentration in the muscles and medium remains unchanged for the next 20 hr as well.

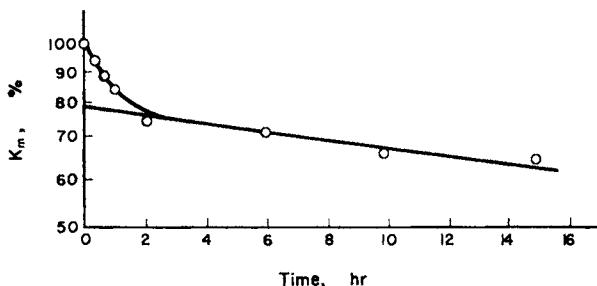


FIG. 102. The lowering of the potassium concentration in frog sartorius muscles during their incubation in potassium-free Ringer's solution.

Along the axis of the ordinates is given the potassium concentration in the muscles ( $K_m$ ) in per cent of the initial concentration (logarithmic scale); along the abscissae—time (Temperature 18–20°).

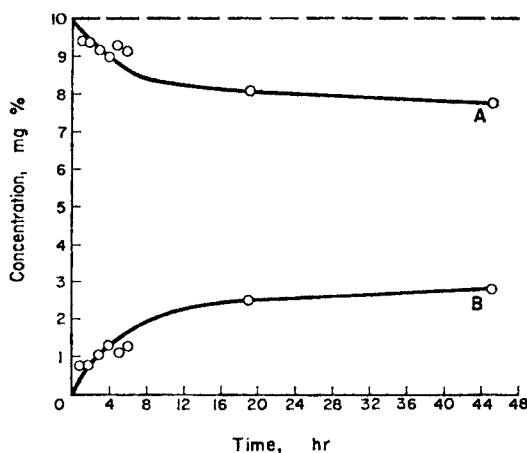


FIG. 103. Calcium loss in frog calf muscles during immersion in potassium-free Ringer's solution.

A—calcium loss in the muscles; B—increase in the calcium concentration in the Ringer's solution.

Thus, muscles are permeable for calcium. It might have been expected that in the course of time its concentration in the muscles and the medium should become the same, but this does not in fact occur. Hence it follows that only a small part of the calcium can escape from the muscle, the remaining, greater, part being firmly bound by the colloids of the muscle tissue and so incapable of diffusion.

According to many reported results, the protoplasm of plant cells, just the same as animal, contains a definite amount of inorganic ions also in the bound state. According to Mason and Phyllis (1936 b), cotton plant cells contain about 25 per cent of their potassium in the bound state, while Ratner (1948) found in the case of barley leaves that, depending on the growth conditions, the corresponding figure ranges from 24·9 to 53·2 per cent. Ratner points out that this part of the potassium is bound labilely with the protoplasmic colloids and that the connection is easily broken down when the tissue is treated with cold water. It is very probable that there is more bound potassium in the solutions than the indicated amount because the quantity was in this case determined from the difference between the potassium in an aqueous extract of ground leaves and the concentration of this cation in cell sap expressed in a press from leaves frozen at  $-16^{\circ}$ . Part of the bound potassium could pass into the solution during this process.

In an analysis of the leaves of various plants Kostytschew and Berg (1929) found that of the total calcium in the leaves from 17·8 to 31·6 per cent should be considered as bound. According to Ratner (1948) there is 74 per cent adsorbed calcium in barley roots and 79 per cent in perilla seeds.

In this connection the work of Mazia (Mazia and Clark, 1936; Mazia, 1938a, b, 1940) is of great interest. In the protoplasm of the cells of water thyme leaves, in yeast cells and in the eggs of sea urchins he discovered that there is a large amount of labilely bound, unionised calcium. Mazia showed that this element is not released from the protoplasm of the cells of water thyme leaves in the course of a 14-day immersion in distilled water; it follows from this that the calcium is bound with the protoplasm. Strontium and barium have similar properties. Potassium and sodium solutions can easily cause the release of calcium from the protoplasm. This indicates that both potassium and calcium are also bound by the protoplasm and can evidently compete with calcium for the same places in which that element is normally bound.

Mazia supposes that a considerable amount of the inorganic ions is bound by the walls of plant cells. In a certain physiological state these ions can be absorbed by the protoplasm. The walls, as Mazia thinks, serve as a reservoir for the supply of salts to the inner contents of the cell. Cations, in his opinion, are bound by the proteins and organic acids in the cell.

The absorption of inorganic substances in considerable amounts by plant cells from very dilute solutions and the inability of these substances to escape into distilled water even during lengthy periods, together with the

capacity shown by some ions for driving others out of a cell (which was discussed in the preceding chapter), also point to the presence of bound ions in the protoplasm. According to Rubinstein, L'vova and Burlakova (1935) calcium and sodium are bound by yeast cells with adsorptional forces.

Thus, on the basis of the results cited it may be asserted that part of the inorganic substances present in resting animal and plant cells is present in the protoplasm bound with the cell colloids. It is especially important to emphasise this in respect of the state of the predominant cation in the protoplasm—potassium.

*The redistribution of ions between cell and medium upon excitation.* Since Overton's time it has been considered that excitation gives rise to a redistribution of inorganic ions between cells and medium. He attached exclusively great significance to the exchange of intracellular potassium for sodium ions from the medium in the process of excitation; however, the role of this exchange has not as yet been elucidated. The essence of this phenomenon and its role in the physiological processes has been discussed from various points of view by numerous workers (Overton, 1902b, 1904; Fenn, 1936, 1940; Nasonov and Aleksandrov, 1943; Hodgkin, 1951, 1958; Katz, 1952a, b, 1956; Belitskii, 1954, 1958; Ungar, 1957; Shanes, 1958, 1960b; Nasonov, 1959a and others).

Information relating to the redistribution of ions upon excitation have, as we shall see below, very great significance for the theory of cell permeability.

The Hungarian physiologist Ernst (1928) discovered that, when frog muscles are excited through the nerves by electrical current, a reduction in their volume occurs. This phenomenon could be explained in such terms as that there occurs in the muscle upon excitation a new growth of ions, around which solvate envelopes are formed (electrostriction). The reduction in volume of the muscle begins before the contraction but at the same time as the action potential. These observations were confirmed in experiments using new and more up to date techniques in a series of papers by Ernst and his colleagues (see Ernst, 1958).

Ernst's conclusions about the transition of potassium into the free state upon excitation of muscles were confirmed by other experiments as well. Thus, Ernst and Fricker (1934a) froze frog gastrocnemius muscles, ground them in the frozen state and then made acetone extractions of potassium; they found that the filtrate from the "snow of resting muscles" yielded  $\frac{1}{2}$ – $\frac{1}{3}$  of the total potassium, whereas the yield from the "snow of muscles" frozen in the contracted state yielded 2–3 times more potassium. Previous excitation of the muscles also leads to an increase of the potassium yield by 1·5 times in comparison with the yield from resting muscles. It follows from this experiment that in the resting state the muscle holds the greater part of its potassium in the non-diffusible (bound) form and that upon excitation or refrigeration part of that potassium is released into the solution.

The same result was obtained by Reginster (1937). The data from his work are shown in Table 91. According to his findings, frog muscle contains potassium precipitable by acetone bound with the proteins in quantities 2·6 to 7·5 times greater than the free potassium, and when the muscle is excited by electrical current either directly or through the nerves, the amount of bound proteins is reduced to about half.

TABLE 91. THE RATIO OF BOUND TO DIFFUSIBLE POTASSIUM IN FROG GASTROCNEMIUS MUSCLES IN THE STATE OF REST AND AFTER EXCITATION WITH ELECTRIC CURRENT  
(after Reginster, 1937)

Experiment	In resting muscle	After direct excitation of muscle	After irritation of muscle through the nerves
1	2·6	1·8	—
2	3·1	1·4	—
3	2·9	1·6	—
4	2·7	—	1·6
5	4·1	—	1·8
6	7·5	—	1·6
Arithmetic mean	3·8	1·6	1·7

Ernst and Schaffer (1928) excited frog gastrocnemius muscles with simultaneous perfusion of the muscles with Ringer's solution and observed that the muscles lost up to 30–50 per cent of their potassium, while in the resting state they lost 6–16 per cent. Ernst and Csúcs (1929) in the same conditions observed release of potassium and phosphate from the muscle into the perfusate and absorption from the medium of sodium and chloride.

Further, Mond and Netter (1930), Ernst and Fricker (1934 a, b) and Netter (1934) in experiments with rabbits found that excitation of the muscles and their perfusion with Ringer's solution led to potassium loss even when the medium contained potassium in quantities in excess of 13 mg per cent, i.e., more than the equilibrium potassium concentration for the muscles. Loss of potassium and absorption of sodium and chloride by frog and rat muscles upon excitation was observed by Fenn (1935) and also Fenn and Cobb (1936).

In experiments with cats Bakhromeyev and Pavlova (1935) established that, upon excitation of muscles through the nerves, the blood flowing from the muscles showed a slight change in calcium content and a very strong change in potassium content. Upon excitation the amount of potassium in the blood plasma and erythrocytes increases and the amount in the muscles decreases. This follows from the data quoted in Table 92.

A detailed study of potassium loss from muscles upon work was made in rabbits by Alekseyeva (1936a, b). The potassium in the rabbit serum was

determined colorimetrically in the resting state and after they had been exhausted on a treadmill. There was observed to be a marked increase in the potassium content of the serum on exhaustion. In other experiments by washing muscles with Ringer's solution containing 10 mg per cent potassium it was discovered that in rest the amount of potassium in the bathing fluid was somewhat reduced; after 10 min excitation of the muscles through the

TABLE 92. THE CHANGE IN THE CALCIUM AND POTASSIUM CONTENT OF BLOOD  
AND MUSCLES IN CATS WITH TIME OF CONTRACTION OF THE MUSCLES  
(from Bakhromeyev and Pavlova, 1935)

Time of taking sample	Calcium in mg%			Potassium in mg%			
	ionised	total		ionised	total		
	plasma	plasma	muscles	plasma	plasma	erythrocytes	muscles
Before contraction	8.9	14.8	8.1	15.6	21.4	499.0	459.0
After and during contraction	8.6	15.6	7.8	17.1	23.0	518.0	413.0

TABLE 93. THE CHANGE IN THE POTASSIUM CONTENT OF RABBIT MUSCLES  
AFTER EXCITATION THROUGH THE NERVES. EXCITATION-10 MIN, REST—15 MIN  
(from Alekseyeva, 1936b)

No. of experiments	Potassium ion concentration in Ringer's solution (in mg%)			
	before washing of muscles	In the washing solution flowing off the muscles		
		before excitation	during excitation	after excitation
10	10.0	9.4	12.3	10.4

nerves there was some increase, and after 15 min rest the amount fell again.

Thus, upon excitation muscle loses a certain amount of potassium reversibly. This is well shown by Table 93.

Kruchakova (1938) in experiments on rabbits observed considerable changes in the inorganic composition of skeletal muscle on lengthy training and fatigue. The training of the muscles was effected by excitation by electric current for 10 min twice a day for 15 days. The muscles (trained and untrained) were fatigued by electric current excitation for 40 min. Training the muscles led to a 23.6 per cent increase in their sodium content and 17.8 per cent and 23.5 per cent increases in their calcium and magnesium contents respectively. At the same time the potassium content in the muscles fell by

12·5 per cent on the average. Fatigue of the muscles caused a very sharp increase in the sodium, magnesium and calcium content. In all these cases the change in the amount of water in the muscles was insignificant.

Indirect data indicating that physiological activity of cells is accompanied by potassium loss from them are produced in several papers by Belitskii (1941, 1950, 1954, 1958), who conducted experiments on isolated frog muscles.

TABLE 94. THE CHANGE IN THE ELECTROLYTE AND WATER CONTENT OF CAT MUSCLES DURING CONTRACTION CAUSED BY EXCITATION THROUGH THE NERVES WITH INDUCED CURRENT  
(in mm or cm<sup>3</sup> per 100 g of dry weight of muscle) (from Tipton, 1938).

Length of excitation (mins)	Potassium	Magnesium	Sodium	Chloride	Calcium	Water
0 (before excitation)	40·2 ± 1·3	2·01 ± 0·25	7·81 ± 0·9	5·54 ± 1·0	0·45 ± 0·1	242·5 ± 12
15	-2·24	--	+2·97	+1·82	--	+15·00
30	-7·2	-0·62	+6·97	+2·25	+0·20	+32·0
45	-5·5	--	+7·1	+2·96	--	+32·0
90	-8·2	--	--	+2·92	--	+10·0
150	-12·4	--	+8·3	+1·82	--	-5·0
180	-12·2	--	+11·4	+1·00	--	-8·0

The minus and plus signs denote, respectively, loss and absorption of the substances by the muscles. 3 hr after excitation no further differences are observed by comparison with the normal state.

The loss of potassium by the muscles upon excitation by electric current (directly or through the nerves) and the absorption by them of chloride, sodium and water has been observed by very many authors: at the same time the rapid reversal of this process, i.e., reabsorption of potassium by the muscles and release of sodium, chloride and water, has been noticed on transition to the resting state. This phenomenon was observed by Fenn and Cobb (1934/5, 1935/6) in experiments with frog and rat muscles, Fenn (1937a, b, 1938b, 1939) in rats and cats, Fenn, Cobb, Manery and Bloor (1938) in cats, Noonan, Fenn and Haege (1941a, b) in rats, Wood, Collins and Moe (1939/40) in dogs and Heppel (1940a) in rats. The last-named author noted that no correlation is observed between the potassium loss by the muscles during excitation and the absorption during this period of chloride and water.

Fenn (1937a, 1938a) attempted to explain the redistribution of ions by supposing that upon excitation of muscles chloride and sodium move only into the extracellular spaces and not into the muscle fibres. He asserted that

upon excitation swelling of the muscles occurs owing to absorption of water by the extracellular spaces. Likewise it is only into these spaces that the sodium and chloride ions from the medium pass together with water. However, a contra-argument against such an assumption is provided by the fact that there is no correspondence observed between the absorption by the tissue of water, chloride and sodium. Another worker, studying this problem after Fenn, Tipton (1938) excited cat calf muscles with induced current through the nerves and observed loss of potassium and magnesium by the muscles and absorption of sodium, chloride and calcium. On short-term excitation there was absorption of water by the muscles, while long-term excitation produced the opposite effect. At the same time the change in the inorganic ion composition did not change direction. The results of these experiments are shown in Table 94.

Radioisotopes have been used to demonstrate that excitation produces a considerable increase in the rate of exchange of intracellular ions for ions of the medium. Thus, Hahn and Hevesy (1941 b) introduced subcutaneously into rats radioactive potassium and after 12 min made part of the rats do 30 min hard work (swimming in a basin). Next both the experimental and the control (non-swimmers) rats were killed and a determination was made of the activity of the potassium in the blood plasma and the skeletal

TABLE 95. THE ABSORPTION OF RADIOACTIVE POTASSIUM BY THE CALF MUSCLE OF RESTING AND SWIMMING RATS (from Hahn and Hevesy, 1941 b).

State of rats	Duration of experiment from time of injection of $^{42}\text{K}$ (min)	% radioactivity of injected dose		Ratio of $^{42}\text{K}$ of muscle to $^{42}\text{K}$ of plasma	% exchange of cell potassium for plasma potassium
		in 1 g of plasma	in 1 g of muscle		
Resting	48	0.080	0.102	1.27	6.7
	49	0.102	0.162	1.59	7.4
	51	0.072	0.122	1.69	7.9
Swimming	47	0.060	0.409	6.82	27.1
	48.5	0.088	0.368	4.17	18.5
	51	0.060	0.492	8.20	39.8

muscle. These data, reproduced in Table 95, show that the amount of muscle potassium that had been replaced by plasma potassium was 4–5 times greater in the swimming rats than in the control. These authors calculate that in 50 min 0.2 mg of potassium penetrate per 1 g of muscle, and a similar amount is released; upon work the potassium exchange is increased fourfold.

Similar results have been obtained by Noonan, Fenn and Haeg (1941 b) in experiments with various rat and frog skeletal muscles: here one muscle

was excited through the nerve (experimental) and its partner not excited (control).

In subsequent experiments with muscles of warm and cold blooded animals using new techniques several authors have shown that excitation produces increases not only in the rate of exchange of intracellular potassium and sodium for the same labelled cations of the medium, but also loss by the muscle fibres of potassium and absorption by them of sodium ions (Born and Bülbring, 1956; Krnjevic and Miledi, 1958; Rayner and Weatherall, 1959; Hurwitz, 1959). This effect has also been demonstrated in experiments with isolated frog muscle fibres (Voronova, 1959; Hodgkin and Horowicz, 1959a). According to Hodgkin and Horowicz (1959a), muscle fibres absorb 15.6 pm/cm<sup>2</sup>/impulse of sodium and lose 9.6 pm/cm<sup>2</sup>/impulse of potassium. Creese, Hashish and Scholes (1958a, b) found that the muscle fibres of rat diaphragm lose 10 pm/cm<sup>2</sup>/impulse of potassium.

Von Euler, von Euler and Hevesy (1946) established by a radioisotope technique that the rate of exchange of sodium of sciatic nerve for sodium of the plasma in cats is increased upon excitation, as also is the rate of exchange of potassium, bromide and phosphate ions.

Flexner and Flexner (1949) observed in embryonic guinea pigs that the ratio of sodium to chloride in the brain tissue increases upon electrical activity of the brain. This shows that upon excitation of the brain there is greater absorption of sodium than of chloride.

According to Cowan (1934) isolated nerves of the crabs *Cancer pagurus* and *Maja squinado* lose a small amount of potassium into sea water. The potassium loss is increased by electrical excitation of the nerve. Release of potassium from the nerve axons during their physiological activity was observed also by Young (1938), Arnett and Wilde (1941), Hodgkin and Huxley (1947, 1953), Abbott, Hill and Howarth (1958), Shanes (1950, 1951) and other workers.

Direct proof of the fact that excitation leads to loss of potassium from the cell and entry into the cell of sodium (and not into the intercellular spaces) can be found in results obtained by a series of workers in experiments with single giant nerve fibres of certain cephalopod molluscs and also with the nerve fibres of other animals.

A graphic impression of this is given by Table 96, which has been made from the data of Keynes (1951b). Using radioactive isotopes this author studied the rate of entry into resting isolated giant nerve fibres of cuttlefish and the release from them into the surrounding medium of potassium and sodium, and also the loss of potassium and absorption of sodium by the nerve upon excitation with electric current.

Upon excitation of the nerve with electric current of a frequency of 100 impulses/sec the influx of potassium by comparison with normal increased 3.3 times, whilst efflux increased 9.1 times. In the same conditions the efflux of sodium increased 22 times and influx 18 times. To sum up, as is

shown by Table 96, each impulse causes the nerves to lose 4.3 pm of potassium and absorb 3.7 pm of sodium.

Similar results have been obtained by many authors. In Table 97, which was composed by Hodgkin (1951), we show the rate of exchange of intracellular potassium for sodium of the medium upon excitation of isolated nerve fibres of various animals by electric current.

TABLE 96. THE EXCHANGE OF POTASSIUM AND SODIUM OF SINGLE NERVE FIBRES OF *Sepia officinalis* FOR THE SAME IONS OF THE MEDIUM. DIAMETER OF THE FIBRES ABOUT  $200\mu$  (from Keynes, 1951 b)

Cation	Rate of movement of cations into the nerve and out of the nerve in the state of rest (in pm/cm <sup>2</sup> /sec)		Loss and absorption of cations upon excitation (in pm/cm <sup>2</sup> /impulse)	
	In	Out	Loss	Absorption
Potassium	16.7	58.4	4.3	—
Sodium	61.0	33.0	—	3.7

According to Shanes (1954a), the isolated giant nerve fibre of the squid loses 3.1 pm/cm<sup>2</sup>/impulse of potassium at 24° and 9.3 pm/cm<sup>2</sup>/impulse at 6°.

Exchange of sodium of the sciatic nerve for sodium of the surrounding saline solution has also been observed in the case of frogs (Asano and Hurlbut, 1956) and of the toad (Shanes, 1954b).

TABLE 97. THE EXCHANGE OF POTASSIUM FOR SODIUM DURING THE ELECTRIC ACTIVITY OF INDIVIDUAL NERVE FIBRES IN pm/cm<sup>2</sup> OF FIBRE SURFACE PER IMPULSE

Animal	Potassium loss	Absorption of sodium	Author
<i>Carcinus maecenas</i>	1.7	—	Hodgkin and Huxley (1947)
<i>Carcinus maecenas</i>	2.5	—	Keynes (1951a)
<i>Sepia officinalis</i>	3.4	—	Weidmann (1951)
<i>Sepia officinalis</i>	4.3	3.7	Keynes (1951 b)
<i>Sepia officinalis</i>	3.6	3.8	Keynes and Lewis (1951 b)
<i>Loligo forbesi</i>	3.0	3.5	Keynes and Lewis (1951 b)
<i>Loligo pealli</i>	—	4.5	Rothenberg (1950)
<i>Loligo pealli</i>	—	4.4	Grundfest and Nachmansohn (1950)

Upon excitation of sympathetic nerve fibres there is observed to be release of a number of organic substances from isolated frog muscles (Tetyayeva, 1938) and of calcium ions in heart muscle (Koshtoyants, Keder-Stepanova and Shidlovskii, 1948).

The release of potassium from frog muscles and onion leaf cells upon excitation with electric current has been observed by Bureau (1934, 1937).

This worker, in accordance with the views of Ernst, sees potassium loss in the active state (excitation) as the results of ionisation of non-diffusible organic potassium compounds.

Mazia and Clark (1936) discovered that in the cell sap of water thyme leaves there is a certain amount of acid potassium oxalate. Analysis of the ash of the leaves of this plant showed that the leaf cells contain a considerable amount of calcium, nonetheless, in normal conditions crystals of calcium oxalate are never observed in the cell sap of these leaves. Short-term exposure of these cells to X-rays, excitation with electric current or 10-min plasmolysis in sugar solution followed by transfer to a normal medium, is, however, sufficient for the very rapid production in the cell sap of an abundant precipitate of calcium oxalate crystals. This experiment shows the existence of a labile compound of calcium with the protoplasm colloids that is capable of transition to the ionised form under the action of external agents.

The experiment with the precipitation of calcium oxalate crystals can be repeated in the same cells if the cells are first placed for a short period in a solution of calcium chloride and then returned to a normal medium for renewed excitation. These experiments are a convincing proof that calcium penetrates plant cells and is bound there by the colloids of the protoplasm.

Ashkenaz (1938) established by irradiation of isolated frog muscle fibres with ultra-violet light that the calcium in them is released from the bound state, becoming able to diffuse into the surrounding medium. According to the observation of Woodward (1949), excitation of frog sartorius muscles by electric current leads to an increase in the exchange of intracellular calcium for that of Ringer's solution. Later, this effect was observed by a number of authors in muscles (Heilbrunn and Wiercinski, 1947; Bianchi and Shanes, 1959; Shanes and Bianchi, 1960; Bianchi, 1960; Shanes, 1960a; Winegrad, 1960; Mullins and Moore, 1960; Winegrad and Shanes, 1962) and in the nerve fibres of invertebrates (Flückiger and Keynes, 1955; Keynes and Lewis, 1956; see also references in Shanes, 1958).

As has already been noted above, there is an abundance of material which shows the release of inorganic phosphorus from cells is continuously effected and is increased upon excitation (see references in Il'yin and Tikhalskaya (1931) and Fenn (1936)). The release of phosphates is observed also in normal and tumour cells, this being increased by the action of damaging agents (Smoilovskaya, 1938; Magat, 1939).

A similar redistribution of electrolytes between cells and their suspending mediums (release of potassium and phosphate, absorption of sodium, chloride and other ions) is observed not only upon excitation, but also under the action of various damaging agents as well as in various pathological states of the organism (Fenn, 1936; Nasonov and Aleksandrov, 1943; Lyubovich, 1947; Zinov'yeva, 1948; Ungar, 1957; Nasonov, 1959a; Troshin, 1960a; Sorokina, 1961 etc.). In the latter cases it is observed that, just as in excita-

tion, the breakdown in the distribution of inorganic substances is reversed upon removal of the damaging factor.

In this connection mention should be made of the very interesting data produced by Roaf and Alderson (1907). They discovered that the action upon erythrocytes, liver, muscles and other organs and tissues of vertebrates of ether, chloroform, carbon dioxide, acetic acid and elevated temperature produces a redistribution of inorganic substances between the cells and the medium. They supposed that the mineral substances of which there is more in the cells than in the medium are bound with the proteins of the protoplasm, and that the action of the above mentioned agents has the effect of breaking down this bond and releasing the substances from the cells.

Thus, the literary data cited provide a convincing demonstration that excitation of the protoplasm results in redistribution of inorganic ions: release from the cell of potassium, phosphate, magnesium, calcium and absorption of sodium and chloride from the medium. When the protoplasm passes from the excited state back to the resting state there is movement of the ions in the reverse direction.

It should, however, be noted that, according to the observations of Ernst and his colleagues, physiological excitation is accompanied by transition of the intracellular potassium from the bound to the free state, but there is no pure loss of potassium and absorption of sodium. Exchange of cellular potassium for sodium of the medium is observed only when the cells are damaged. They consider that this is valid for other ions too (Ernst, 1958; Niedetzky, 1959; Tigyi, 1959a, b, c; see also Mullins, 1954).

The majority of workers treat this phenomenon of the redistribution of ions upon excitation in the spirit of the classical membrane conception and see its cause in the fact that upon excitation the semi-permeability of the protoplasmic membrane is destroyed.

However, if a stand is made on the classical membrane theory of permeability, it is completely impossible to account for the causes of the return motion of ions after excitation of cells in the opposite direction – “against the concentration gradient”.

These difficulties of explaining the distribution of ions between cells and medium are discussed by Fenn in his review (Fenn, 1936). In the course of recounting the factual data on muscle electrolytes he frequently noticed that these are contradictory to the membrane conception and he expressed a desire to develop a different, more satisfactory theory. Fenn concluded his article with the following words: “Thus, according to the theory, muscle in normal conditions should be impermeable for electrolytes. A change in the electrolyte content should be considered as a functional change in the permeability of the membrane. This theory has advantages in its great flexibility, but it contains very many obscure elements and really acts merely as a screen behind which we hide our ignorance” (p. 482).

Of course, the return to normal ion distribution after a period of

excitation can be accomplished by a cell only at the expense of a certain amount of energy, which can be derived only from metabolism. This energy also provides the means for the movement of ions in the direction of their "greater concentration". Both the opponents and the adherents of the membrane theory are in agreement on this point.

All these phenomena connected with the distribution of inorganic ions and with their redistribution at the moment of excitation find a simple and, in my opinion, correct explanation from the standpoint of those writers who recognise the existence in the protoplasm of phase properties and see the decisive role in the mechanism of cell permeability played by sorption and not by diffusion through the cell membrane.

The sorptional mechanism which maintains the characteristic ion distribution for resting and excited protoplasm provides a good explanation of many facts, among them the following: (1) while cells are indubitably permeable for potassium, sodium, chloride, magnesium, phosphates and other ions, their concentrations inside and outside the cell are not equal; (2) the rapid release of sodium and chloride from a cell and the entry of potassium into it when the cell moves from the excited state to the resting state; (3) the fractional release of potassium, sodium and chloride while tissues are kept in artificial media; (4) the existence of different equilibrium levels of concentrations in the protoplasm and the movement of ions "against the concentration gradient"; (5) the increase in the fraction of diffusible potassium in muscles after their excitation and so on (see Nasonov and Aleksandrov, 1943).

The transition of the protoplasm from one state to another (rest—excitation—rest), which is accompanied by a change in the state of the inorganic substances in the cell and their redistribution, is a process that can occur only with the participation of the reactions of intracellular metabolism. It requires a stream of definite biochemical conversions of substances yielding energy and raw material for this process. On the other hand, the inorganic substances themselves in these biochemical and physio-chemical reactions evidently play a direct and very important role. The part played by ions in the transition of the protoplasm from one state to another has attracted the attention of only a few scientists (Nasonov and Aleksandrov, 1940, 1943, 1945; Szent-Györgyi, 1948; Koshtoyants, 1947, 1951; Heilbrunn, 1952, 1956; Nasonov, 1959a, etc.). Thus, Nasonov and Aleksandrov (1943) wrote the following: "The liberation of substances during excitation is in the membrane conception only a kind of side effect connected with the unnecessary and unintelligible waste of the materials which leave the bounds of the cell; but as we understand it, their liberation acquires deep significance as a special kind of trigger mechanism regulating the correct flow of the chemical conversions required at the given moment. From the membrane point of view we have at the moment of excitation only the emission of substances soluble in the protoplasm, while from our point of view it is a matter of their transition to the active state" (p. 597).

This point of view was developed by Koshtoyants (1947, 1951). He cites many convincing facts in support of the proposition that there lies at the basis of excitation the conversion of proteinaceous bodies, as a result of which calcium, potassium and other ions are liberated from the state of bondage with the proteins, these ions being the factors determining the start and regulation of the course of certain enzymatic reactions. As he sees it, "it is possible that acetylcholine, which is liberated in nervous excitation, can, by the stimulation of proteolysis, facilitate the release from the above mentioned compounds of ions of calcium, magnesium and potassium, which in their turn cooperate in the subsequent setting in motion of the enzymatic reactions that lie at the foundations of the reversible structural changes of the contractile substance of muscle – myosin" (p. 63).

A similar position in this discussion is taken by Kometiani and his colleagues (Kometiani, Dolidze and Klein, 1944; Kometiani, Klein and Dolidze, 1946; Kometiani, 1947, 1948a, b). They observed that during the excitation of muscle fibres part of the potassium is split off and goes into a form that is capable of diffusion. The direct cause of this is, in the opinion of these writers, the action of acetylcholine on the proteins, which under its action change their physico-chemical properties in such a way that part of the potassium bound with them is released and goes into solution.

Heilbrunn (1928, 1930, 1952, 1956) developed the widely known "coagulatory" theory of irritation and excitation. His idea was that the action on the cell of an irritant or damaging agent causes the liberation from the bound state in the surface layer of the protoplasm of calcium, which, on penetrating the cell, causes the protoplasm to coagulate. This process, so he supposes, is similar to the process of the coagulation of blood. Much interesting information on the part played by the liberated calcium in excitation is also to be found in the work of other scientists (see Detlaf, 1957, 1958a, b; Detlaf and Turpayev, 1957, etc.). Fundamental criticism of the coagulatory theory of excitation and damage of the protoplasm is to be found in the work of Nasonov and Aleksandrov (1940) and Nasonov (1959a).

Thus, the problem of the state of inorganic ions in the protoplasm acquires a new and deep significance, since the distribution of inorganic substances between cell and medium is connected with the state of the live matter itself during periods of rest and activity alike and with those biochemical processes that form the basis of the physiological activity of the protoplasm.

### *3. The Role of the Sorptional Factors in the Mechanism of the Distribution of Inorganic Substances between Cells and Medium*

In discussing the problems of the permeability of cells, many scientists have come to the conclusion that there lies at the basis of the penetration of non-electrolytes and electrolytes into the cell, and also of the distribution of these substances between cells and medium, a single general mechanism (Naso-

nov and Aleksandrov, 1937, 1940, 1943; Aleksandrov, 1939 a; Nasonov, 1939; Sabinin, 1940, etc.). Thus, Sabinin (1940), considering the laws of the permeability of cells for non-electrolytes and electrolytes, wrote that "... the problem of the passage of substances into the cell can be considered from a single overall view point" (p. 82).

Because the basic role in the process of the passage into the cell of non-electrolytes (alcohols, sugars, urea, creatinine etc.), of a number of organic acids (ascorbic, pyruvic, uric and other acids), of amphoteric electrolytes (amino acids) and organic electrolytes of high molecular weight (vital dyes) is, as we have seen, played by sorption factors (the solubility of substances in the protoplasm, their adsorptional and chemical interaction with the colloids of live matter), it is natural to suppose that these same factors should also be of vital significance in the process of the entry into cells of inorganic substances and also in the mechanism of their asymmetrical distribution between cells and medium.

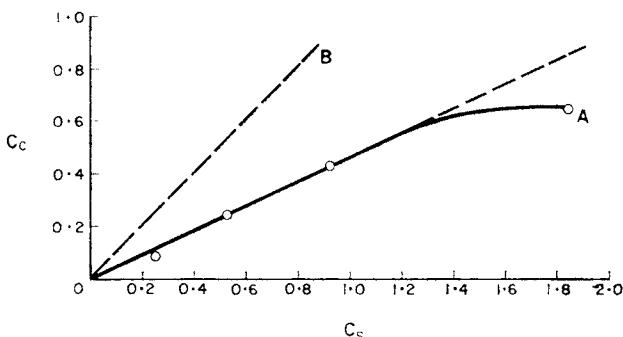


FIG. 104. The dependence of the chloride ion concentration in yeast cells ( $C_c$ ) on the concentration in the medium ( $C_s$ ).

Let us now consider the significance of each sorptional factor separately in the process of the distribution of mineral ions between cells and medium. This problem has formed the subject of my own papers, in which a study was made of the distribution of chloride ions between yeast cells (*Saccharomyces ellipsoideus*) and the surrounding solution (Troshin, 1948 a).

In Fig. 104, the curve OA shows the relation between the chloride ion concentration in the yeast cells ( $C_c$ ) and the concentration in the surrounding medium ( $C_s$ ) in conditions of diffusion equilibrium.

The broken line OB reflects equality of chloride concentration in the yeast and in the medium, such as would occur if chloride were as soluble in the protoplasm as in the water of the surrounding medium and were not adsorbed by the colloids of the protoplasm. The chloride ion distribution curve OA, which was obtained experimentally, starts from the origin, runs for a considerable distance below the broken line and indicates a linear relation

between  $C_c$  and  $C_s$ . It corresponds to the formula obeyed by the distribution of sugar and other substances, namely:  $C_c = C_s K + A$ .

This formula, which reflects the dependence of the non-electrolyte content in a cell on the concentration in the medium, should be supplemented somewhat when applied to the phenomenon of ion distribution. It is known from physical chemistry that the magnitude of the distribution coefficient ( $K$ ) of the cation or anion of a salt between two phases is determined not only by the solubility in them of one ion (the cation or anion), but also by the solubility of its partner with which it is electrostatically bound.

The amount of ion adsorbed ( $A$ ) will be determined not by Langmuir's formula, which is valid for the adsorption of non-electrolytes (for non-polar adsorption), but by a formula developed by Ye. Gapon (see Gapon and Gapon, 1948; Nikol'skii, 1948; Kablukov, Gapon and Grindel', 1935). Gapon's formula reflects the process of polar (exchange) adsorption. In this case the amount of the ion adsorbed will be equal to the amount of the ion emitted which was earlier adsorbed by the colloids of the live matter.

Further, the ions passing into a cell—at any rate some of them—can become bound chemically with the protoplasm colloids. Consequently, if the amount of the ion bound owing to exchange adsorption in the cell be denoted by  $A_a$ , and the amount of the ion bound chemically by  $A_c$ , then the total bound ion content in the protoplasm will be  $A = A_a + A_c$ , and the limiting amount of bound ion  $A_\infty = A_{a\infty} + A_{c\infty}$ .

Thus, the formula reflecting the process of the sorption of inorganic ions by live matter can be written thus:  $C_c = C_s K + A_a + A_c$ ; and in the case of limiting adsorption and chemical binding,  $C_c = C_s K + A_{a\infty} + A_{c\infty}$ , or  $C_c = C_s K + A$  and  $C_c = C_s K + A_\infty$ , respectively, where  $C_c$  is the ion concentration in the cell, and  $C_s$  that in the medium. The two latter formulae are similar to those reflecting the sorption of non-electrolytes by cells.

Figure 104 shows that the solubility of chloride ions in the protoplasm of yeast cells is less than half (55 per cent less) that in the water of the surrounding solution ( $K = 0.45$ ) and that they are not bound by the colloids of the protoplasm; the chloride is present in the yeast cells only in the dissolved state ( $A = 0$ ).

The deviation from linearity of the dependence at high concentrations of this ion in the medium is probably due to the fact that the cells lose much water in these conditions. This phenomenon can be explained in the same way as in the above described cases of the distribution of lactose between yeast cells and medium and of sodium sulphate between coacervate and equilibrated liquid; upon dehydration of coacervates and the protoplasm they release the "loosely bound water", as a result of which the solubility of substances in the remaining water of the protoplasm is lowered.

The applicability of this formula for ion distribution has been discovered in experiments with a variety of biological subjects. The following data may be adduced in confirmation of this.

Detailed studies of the dependence of the chloride ion concentration in muscles on the concentration in the medium in conditions of diffusion equilibrium was made by Fenn and his colleagues, Steinbach and other scientists. Thus, Fenn, Cobb and Marsh (1934/5) in an already quoted paper

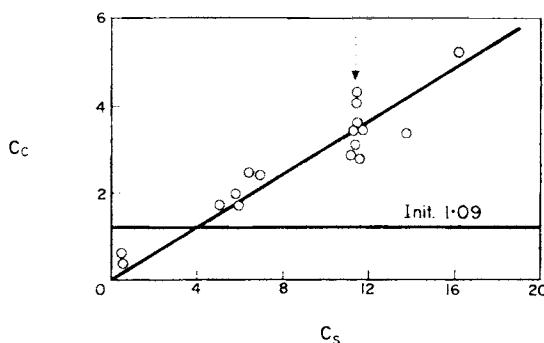


FIG. 105. The dependence of the chloride ion concentration in frog muscles ( $C_c$ , in mequiv/100 g of tissue) on the concentration in the medium ( $C_s$ , in mequiv per cent). (From Fenn, Cobb and Marsh, 1934/5).

The arrow indicates the chloride concentration in muscle lying in Ringer's solution. Init. is the concentration of chloride in the control muscle (immediately after extraction from the organism).

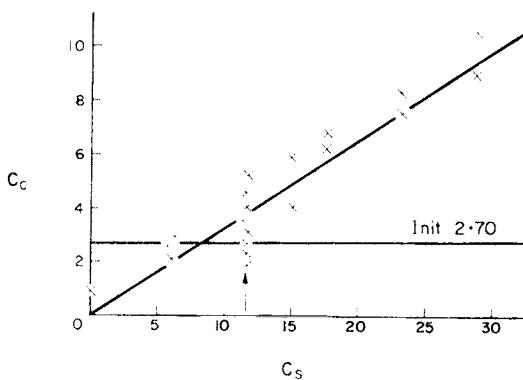


FIG. 106. The dependence of the sodium ion concentration in frog muscle ( $C_c$ , in mequiv/100 g of tissue) on its concentration in the medium ( $C_s$ , in mequiv per cent). (From Fenn, Cobb and Marsh, 1934/5).

studied the distribution of sodium and chloride ions between isolated frog sartorius muscles and a surrounding isotonic solution in which the chloride and sodium concentrations were varied within fairly wide limits. In Figs. 105 and 106 we show the respective distribution curves for chloride and sodium ions.

As can be seen in these figures, chloride ions are absorbed by the muscle up to 31 per cent of the external concentration, while sodium ions are up to 33 per cent for any concentration of these ions in the surrounding medium.

If the results of the experiments of Fenn and his colleagues are worked out in the same way as was done in my experiments studying the distribution of sugars, dyes and other substances, there is no difficulty in finding a similarity in the distribution of these ions with the distribution of other substances.

The results of the experiments of these authors, calculated relative to the water of the muscle fibres, are shown in Fig. 107. It is easy to see that we have here a linear relation of the sodium and chloride ion concentrations in the muscle fibres to their concentrations in the medium; the straight line corresponding to the sodium distribution does not begin at the origin of co-ordinates, as was arbitrarily shown in Fig. 105 by Fenn and his colleagues,

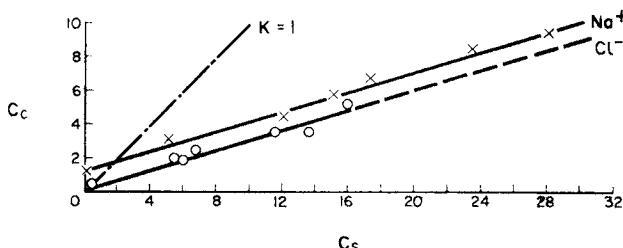


FIG. 107. The dependence of the concentration of sodium and chloride ions in muscle fibres ( $C_c$ , in mequiv per cent in the water of the muscle fibres) on their concentration in the medium ( $C_s$ , in mequiv per cent). (From Fenn, Cobb and Marsh, 1934/5).

but somewhat higher; when  $C_s = 1.6$  mequiv per cent it cuts the bisector and for a considerable distance runs below it. Thus, when  $C_s < 1.6$  mequiv per cent the sodium concentration in the muscle fibre is greater than in the medium ( $Q > 1$ ), while when  $C_s > 1.6$  mequiv per cent, it is less ( $Q < 1$ ). The sector on the ordinate formed by its intersection with the sodium distribution line corresponds to the amount of the latter which is firmly bound by the muscle colloids and does not come out when the tissue is placed for 5 hr in a medium free of this ion. If we now draw a line from the origin parallel to the sodium distribution line, then this line will reflect the dependence of the dissolved amount of sodium ions in the sarcoplasm on their concentration in the medium. This, as can be seen from the figure, corresponds to the chloride ion distribution.

Thus, the distribution of sodium and chloride ions follows the same law as is observed in experiments with non-electrolytes, dyes and other substances. The formula:  $C_c = C_s K + A$  is applicable here too. In the present case  $A$  is constant over the whole range of concentrations tested. For sodium it is 1.2 mequiv per cent for the quantity of dry residue of muscle correspond-

ing to 100 ml of intracellular water, and for chloride it is zero, that is, chloride is not bound at all by the cell colloids. This is confirmed in numerous works in which it is shown that chloride is completely lost from muscle into a chloride-free solution. The coefficient  $K$  in the formula is 0.3 for sodium and chloride. In other words, sodium and chloride ions are present in muscle in the dissolved state in equivalent quantities, and the certain excess of sodium over chloride is due to the fact that a certain amount of the sodium is bound.

TABLE 98. THE CONCENTRATION OF EXCHANGING SODIUM IONS IN FROG SARTORIUS MUSCLES AT VARIOUS CONCENTRATIONS OF THIS CATION IN THE MEDIUM. TEMPERATURE 18–20°.

No. of expts.	Concentration of cation in medium (mequiv%)	$Q = \frac{\text{counts/min/g of fresh muscle}}{\text{counts/min/1 ml of medium}}$	Concentration of free sodium in muscle (in mequiv/100 g of fresh tissue)	Concentration of free sodium in muscle fibres (mequiv/100g of intracellular water)
4	11.11	0.31	3.44	3.33
9	8.33	0.31	2.58	2.49
5	5.56	0.32	1.78	1.74
5	2.78	0.33	0.92	0.91
5	1.39	0.35	0.49	0.50

These calculations are completely confirmed by data obtained by myself in a study of the distribution of radioactive sodium between frog sartorius muscles and Ringer's solution (Troshin, 1957).

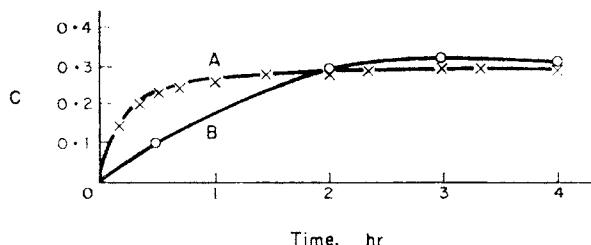


FIG. 108. The change with time of the ratio of the concentration of radioactive sodium in frog muscles to its concentration in Ringer's solution.

A—in normal Ringer's solution with added  $^{24}\text{Na}$ ; B—in Ringer's solution + sucrose with added  $^{24}\text{Na}$  (concentration of  $\text{NaCl} = 13.9\text{ mM}$ ). Temperature 18–20°.

In Table 98, we show the ratios  $Q$  of the  $^{24}\text{Na}$  concentrations in the muscles to its concentration in the medium which contained in solution 11.11, 8.33, 5.56, 2.78 or 1.39 mequiv per cent of sodium with an admixture of  $^{24}\text{Na}$ . The missing amount of sodium was replaced by sucrose. The duration of the storage of the muscles in these solutions was 3 hr; in this time complete

diffusion equilibrium was attained in all the solutions, as can be seen from Fig. 108. The data quoted in the table are the arithmetic mean of 4–9 experiments.

It follows from the table that  $Q$  changes little with change in the sodium chloride concentration in the medium.

It is possible that even this small increase in  $Q$  upon decrease in the sodium concentration in the Ringer's solution is connected with the exchange of a small amount of labile bound muscle potassium.

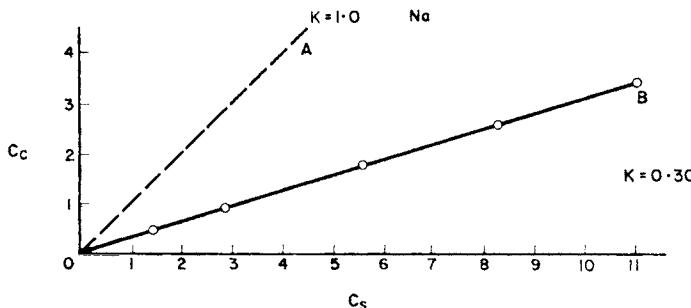


FIG. 109. The distribution of the free fraction of sodium between frog sartorius muscle fibres and Ringer's solution, in which part of the sodium has been replaced by galactose ( $C_s$ , in mequiv per cent,  $C_c$ , in mequiv per 100 g of water).

The values of  $Q$  quoted in the table have been used as a basis for calculating the concentration of free (exchanging for  $^{24}\text{Na}$ ) sodium in the muscles and muscle fibres. The concentrations of such sodium in the muscle fibres are expressed in mequiv per 100 g of intracellular water. These calculations show that with decreasing sodium concentration in the surrounding medium a proportional decrease occurs in its concentration in the muscle fibres. This can be seen clearly from Fig. 109, in which is shown the distribution of free sodium between muscle fibres and medium.

If the sodium concentration in the water of muscle fibres were the same as in the surrounding medium, then the sodium distribution would correspond to the line OA. In fact it corresponds to the straight line OB, which runs below the bisector and obeys the law:  $C_c = C_s K$ , where  $K = 0.30$ . In other words, there is 70 per cent less free sodium in the muscle fibres than in the medium at all tested concentrations of this ion.

Thus, chemical analyses of the content of muscles and medium in the presence in them of sodium and the study of the distribution of  $^{24}\text{Na}$  between muscles and Ringer's solution indicate the same, namely:

1. Sodium ions unquestionably penetrate muscle fibres and escape from them outwards. This penetration of sodium occurs even when there is no change in the concentration of this cation in the muscle and medium as a whole.

2. The basic mass of the muscle sodium is dissolved and capable of diffusion. The concentration of such sodium in the water of the muscle fibres is about 30 per cent of the external sodium concentration.

3. About 20 per cent of the muscle sodium is the bound fraction of this cation. This amount of sodium is not capable of diffusion.

For other live tissues the bound sodium fraction can be either greater or less than in the case of muscle. Thus, Abelson and Duryee (1949), using radioactive sodium, discovered that only 12 per cent of the sodium of frog's eggs is capable of exchange for sodium of the medium; this amount of exchange is reached in the first 30–60 min and then remains unchanged for many hours. Autoradiographs of egg sections showed that the  $^{24}\text{Na}$  penetrating the cells is uniformly distributed in the whole protoplasm. They established by chemical analysis that the sodium concentration in the egg cells is 0·082 per cent of the dry weight of the eggs.

The distribution of sodium and chloride ions in experiments with frog sciatic nerve was studied by Fenn, Cobb, Hagnauer and Marsh (1934/5). These authors' results are shown in Figs. 110 and 111, from which it can be seen that the concentration of sodium and chloride ions in the nerve changes in direct proportion to the change in the concentration of these ions in the surrounding solution. At all the tested concentrations of sodium and chloride the frog nerve contained 57 per cent of these ions as compared with their external concentration (calculated in relation to the fresh weight of the nerve). The authors came to the conclusion that sodium and chloride ions penetrate only the extracellular spaces and do not penetrate the nerve fibres.

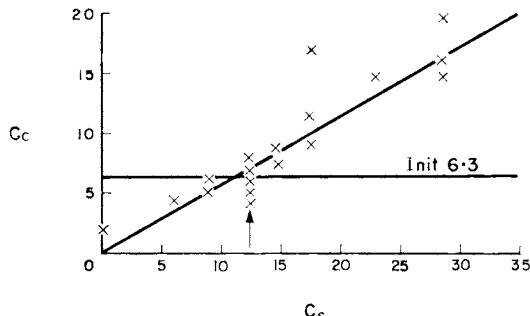


FIG. 110. The dependence of the sodium ion concentration in frog sciatic nerve ( $C_c$ , in mequiv/100 g of nerve) on its concentration in the surrounding medium ( $C_s$ , in mequiv per cent). Length of experiment — 5 hr. (From Fenn, Cobb, Hagnauer and Marsh, 1934/5).

Hence it follows that the extracellular space of the nerve is equal to 57 per cent. Such an interpretation is evidently incorrect. If the extracellular spaces were really so large, then it is unintelligible that the nerve normally contains 3·7 mequiv per cent of chloride and after 5 hr in Ringer's solution the

chloride content is almost doubled (to 6.6 mequiv per cent), whereas the initial sodium content remains substantially unchanged even after 5 hr in Ringer's solution at a value of about 6.3 mequiv per cent. If the "intercellular space" of the nerve is determined from the normal chloride content of the nerve on the basis that it is all in the intercellular spaces, then it will be equal not to 57 per cent of the weight of the nerve, but to about half of this value.

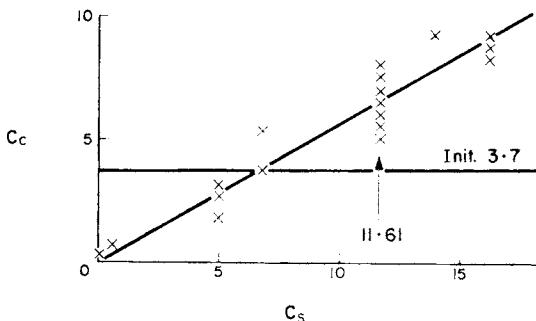


FIG. 111. The dependence of the chloride ion concentration in frog sciatic nerve ( $C_c$ , in mequiv per 100 g of nerve) on its concentration in the surrounding medium ( $C_s$ , in mequiv per cent). Length of experiment—5 hr. (From Fenn, Cobb, Heginauer and Marsh, 1934/5).

The results of these experiments with frog nerves, by analogy with the preceding data, give yet more grounds for speaking of the penetration of sodium and chloride ions into nerve fibres, their distribution between nerve and medium being determined principally by the solubility of these ions in the axoplasm, which is considerably lower than their solubility in the surrounding medium.

We cite above data showing that lithium ions cannot completely expel sodium from muscles. In the state of diffusion equilibrium the lithium concentration in muscle fibre remains lower than the earlier free sodium concentration in the fibre (Troshin, 1961). We further determined by means of the flame spectrophotometer the equilibrium concentration of lithium ions in the muscle fibres. Isolated frog sartorius muscles were immersed sufficiently long to establish diffusion equilibrium (for 3 hr) in "Ringer's solutions" containing a certain amount of sodium replaced by lithium. The results obtained are shown in Fig. 112. This figure shows that lithium ions are distributed between muscle and medium in the same way as chloride ions and free sodium: the lithium ions are not bound by the muscle colloids. However, the distribution coefficient  $K$  for lithium is lower—equal to 0.21.

The distribution of potassium has been the subject of much work. The analysis of the results of these studies shows convincingly that the distribution of potassium is effected by the same mechanism, though, in contrast to

sodium and chloride ions, the dissolved part of the potassium in the protoplasm forms an insignificant fraction of the whole.

Thus, Steinbach (1940b) produces some interesting data on the distribution of potassium between muscles and the surrounding medium. He immersed frog sartorius muscles in Ringer's solutions or isotonic sugar solutions containing various concentrations of potassium and kept them there until diffusion equilibrium was established at 22° (5–6 hr) or at 3–4° (20 hr). Then the potassium content in the muscles was determined. The results of Steinbach's experiments are shown in Fig. 113, while in Fig. 114 they are shown recalculated relative to the intracellular water. The curve OB in the figure depicts the dependence of the potassium concentration in the muscles ( $C_c$ ) on its concentration in the surrounding fluid ( $C_s$ ). The form of the curve shows that the dependence of  $C_c$  on  $C_s$  obeys the same law as the distribution of sugars, creatinine, alanine and vital dyes:  $C_c = C_s K + A$ . The curve OB

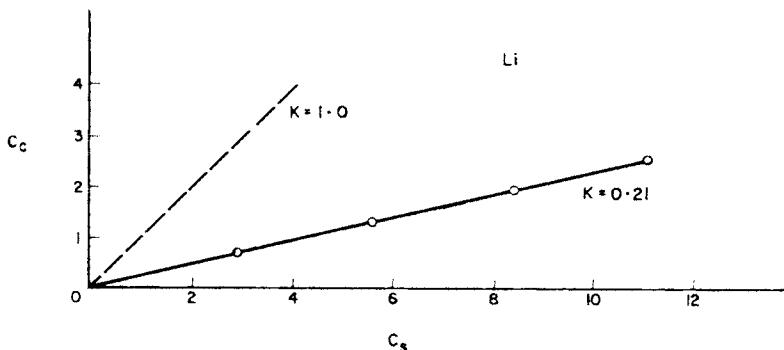


FIG. 112. The distribution of lithium ions between frog sartorius muscle fibres and Ringer's solution, part of whose sodium has been replaced by lithium.

$C_s$ —the concentration of lithium in the medium (mequiv per cent);  $C_c$ —the concentration of lithium in the muscle fibres (mequiv/100 g of cell water). Temperature 18–20°.

from  $E$ , i.e. for  $C_s > 0.5$  mequiv per cent, shows linear dependence of  $C_c$  on  $C_s$ . This means that the quantity  $A$  at these potassium concentrations in the medium becomes a constant ( $A = A_\infty$ ) without change for further increase in  $C_s$ . This is the limiting amount of potassium which the muscle colloids can bind chemically or adsorptionally. As shown in Fig. 114, this is equal to the sector on the ordinate OE, that is, 12.0 mequiv per cent. The line OC, parallel to EB, reflects the dependence of the concentration of dissolved potassium in the muscle fibre ( $C$ ) on  $C_s$ . This straight line corresponds properly to the distribution of the potassium ions between two phases: the aqueous surrounding medium and the sarcoplasm. In this case the distribution coefficient  $K = 0.45$ , i.e., the solubility of this cation in the sarcoplasm is 55 per cent less than in the water of the equilibrated liquid. The line OD represents the case  $K = 1$ .

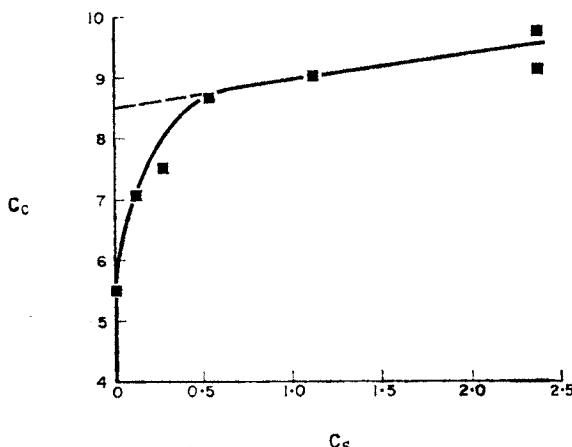


FIG. 113. The dependence of the potassium ion concentration in frog muscles ( $C_c$ , in mequiv/100 g of muscle) on its concentration in the surrounding medium ( $C_s$ , in mequiv per cent). (From Steinbach, 1940a).

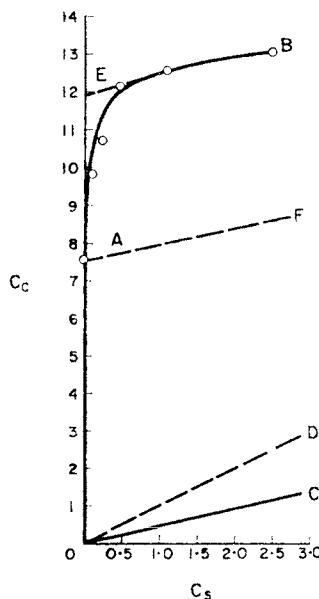


FIG. 114. The dependence of the potassium ion concentration in muscle fibres ( $C_c$ , in mequiv per cent) on the concentration of these ions in the surrounding medium ( $C_s$ , in mequiv per cent). (From Steinbach, 1940b).

The potassium bound in the muscle can in its turn easily be divided into two parts differing in the form of their bond with the cell colloids, one part probably being in labile bond with the colloids. This is, in all probability, the adsorbed potassium. The other part is more firmly bound. The amount of such potassium corresponds in the figure to the sector OA on the ordinate. This potassium does not leave the muscle even after long periods in potassium-free solutions. In Fig. 114 the amount of firmly bound potassium in the muscle fibres, whose content is independent of the concentration of this ion in the medium, is denoted by the broken line AF, which runs at the level of 7.6 mequiv per cent above OC.

Thus, it follows from the analysis of Steinbach's data that, per 100 ml of intracellular water, muscle contains 4.4 mequiv per cent as the "limiting amount of adsorbed potassium"  $A_\infty$ , 7.6 mequiv per cent of "chemically bound" potassium  $A_{c\infty}$  and dissolved potassium  $C$  to the extent of 45 per cent of the external equilibrated concentration of this ion. When  $C_s = 2.5$  mequiv per cent, the amount of dissolved potassium in the muscle fibres is equal to about 1.13 mequiv per cent.

The above mentioned work of Steinbach (1937) provides further information which we must mention: this was obtained in a study of the potassium ion distribution between isolated retractors of the holothurian *Thyone briareus* and sea water. In Fig. 115 we show the distribution of potassium ions after 4–6 hr immersion of the retractors in sea water containing various potassium

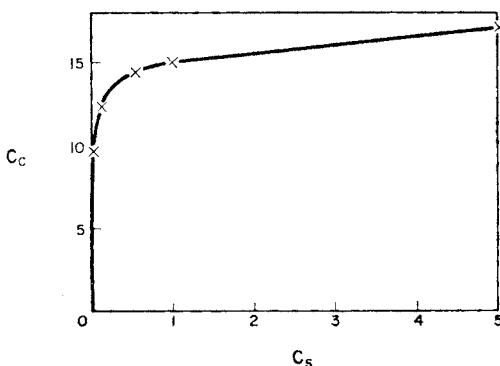


FIG. 115. The dependence of the potassium ion concentration in holothurian retractors ( $C_s$ , in mm 100/cm<sup>3</sup>) (from Steinbach, 1937).

concentrations. Steinbach's results have been reworked relative to the intracellular water, taking into account that the extracellular space of the retractors is 17.8 per cent. The reworked results are given in Fig. 116.

The path of the potassium distribution curve shows clearly that the potassium in the muscle fibre is present in three different states, as in the case of frog muscles: (1) potassium firmly bound by the protoplasm colloids—

$A_c = 14.0$  mequiv per cent (this potassium does not leave the muscles even in a potassium-free medium), (2) potassium labilely bound by the colloids ( $A_a$ ). The limiting amount of such potassium which the muscles can bind,  $A_{\infty}$ , is 6.9 mequiv per cent (in a potassium-free medium this potassium is released from the muscle), (3) potassium dissolved in the sarcoplasm — C. The

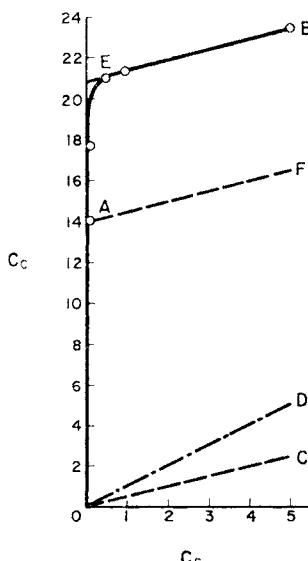


FIG. 116. The dependence of the potassium ion concentration in the muscle fibres of the holothurian ( $C_c$ , in mm per 100 ml of intracellular water) on their concentration in sea water ( $C_s$ , in mm per 100 ml) (from Steinbach, 1937).

amount of this last fraction in the muscle changes directly proportionally to the change in its concentration in the external medium with distribution coefficient  $K = 0.48$ . In other words, 52 per cent less potassium is dissolved in the muscle sarcoplasm than in the water of the surrounding medium.

The published data relating to the distribution of potassium between nerves and the surrounding liquid leaves no room for doubt but that in this case, as in the case of muscles, the cation distribution is regulated by the same factors. I quote here only one meticulous study by Fenn, Cobb, Hegnauer and Marsh (1934–5). These workers placed isolated nerves of various animals in Ringer's solutions or sugar solutions containing different concentrations of potassium and determined the amount of this cation present after 5 hr. Some of their data are shown in Figs. 117 and 118.

These figures show the dependence of the potassium content in the nerve of the frog, crab and crayfish in relation to the concentration of this cation in the surrounding liquid after 5 hr immersion of the nerve at 20–22°. As can

be seen from the figures, there is a linear relation between the potassium concentration in the nerve ( $C_c$ ) and its concentration in the medium ( $C_s$ ). When  $C_s = 0$ , more than half of the original amount of potassium remains in the nerve of the frog and crab.

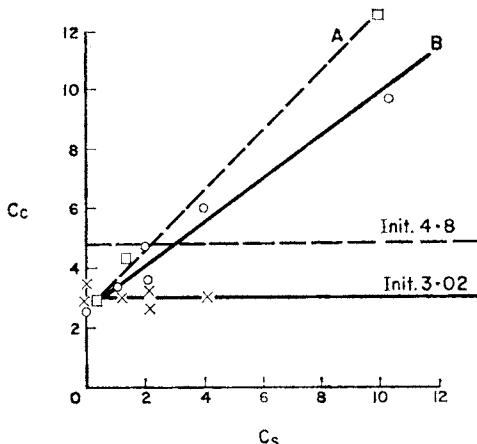


FIG. 117. The dependence of the potassium ion concentration in frog nerve ( $C_c$ , in mequiv/100 g of nerve) on their concentration in the surrounding medium ( $C_s$ , in mequiv per cent). Length of experiments — 5 hr (from Fenn, Cobb, Hagnauer and Marsh, 1934/5).

A—nerves of spring frogs; B—nerves of autumn frogs.

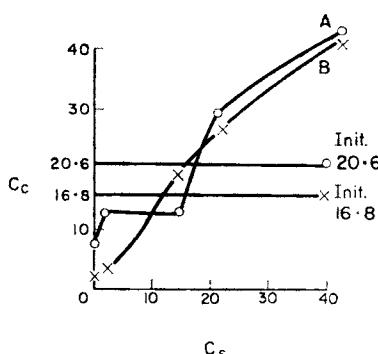


FIG. 118. The dependence of the potassium ion concentration in crab nerves (A) and crayfish nerves (B) ( $C_c$ , in mequiv/100 g of nerve) on their concentration in the surrounding medium ( $C_s$ , in mequiv per cent). Length of experiments — 5 hr (from Fenn, Cobb, Hagnauer and Marsh, 1934/5).

It is interesting to note that "March" frogs differ considerably in their nerve potassium content from "November" frogs. "March" frogs have 4.8 mequiv/100 g of nerve, "November" frogs 3.02 mequiv, the amount of firmly bound potassium (not released from the nerve when  $C_s = 0$ ) being

about the same in both cases—somewhat less than 3.02 mequiv/100 g of fresh nerve.

Unfortunately, Fenn, Cobb, Hegnauer and Marsh (1934/5) made an insufficient number of experiments with small potassium concentrations, and it is therefore difficult to determine the relative amounts dissolved and bound in the nerves. In one series of experiments with small concentrations the authors obtained the following data, shown in Fig. 119.

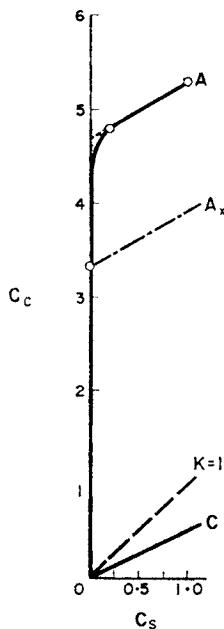


FIG. 119. The dependence of the potassium ion concentration in frog nerve ( $C_c$ , in mequiv/100 g of nerve water) on their concentration in the surrounding medium ( $C_s$ , in mequiv per cent) (from Fenn, Cobb, Hegnauer and Marsh, 1934/5).

The figure shows that in these conditions the potassium firmly bound in the nerve  $A_c$  is 3.3 mequiv/100 ml of nerve water, the amount of dissolved potassium ( $C$ ) about 50 per cent of the external concentration ( $C = C_s K$ , where  $K = 0.5$ ). This calculation is very approximate, if only for the reason that the intercellular spaces, whose size is unknown, have not been taken into account. However, these data are a convincing demonstration that the amount of labile and firmly bound potassium in nerve ( $A_a + A_c$ ) is considerably greater than the dissolved.

Fenn and Haege (1942) also studied the distribution of magnesium ions between frog muscles and Ringer's solution containing from 0 to 6 mm per

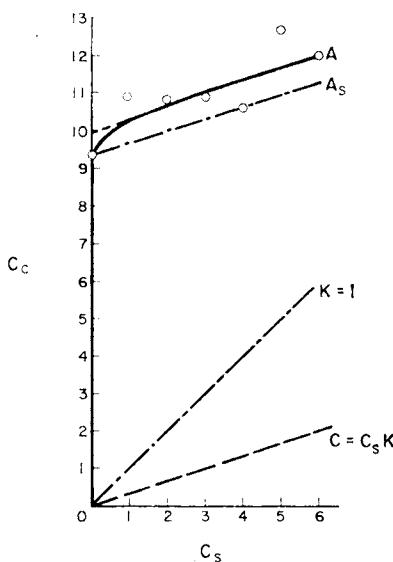


FIG. 120. The dependence of the magnesium ion concentration in frog muscles ( $C_c$ , in mm) on the concentration of this cation in the medium ( $C_s$ , in mm) (from Fenn and Haege, 1942).

litre of this cation. The solution had a phosphate buffer, the experiments lasting 5 hr.

The results of these experiments calculated relative to the intracellular water are set out in Fig. 120. As can be seen from the path of the magnesium distribution curve, the great bulk of this is found in muscle in the firmly bound form— $A_c$ . There is 9.04 mm/l of intracellular water of such magnesium in muscle. The limiting amount of adsorbed magnesium  $A_{\infty}$  is 0.96 mm/l of intracellular water and of dissolved magnesium ( $C = C_s K$ ) about 35 per cent of the external concentration ( $K = 0.35$ ). Thus, the magnesium ion distribution very closely recalls the potassium distribution. Here too the basic mass of the magnesium is in the firmly bound and labilely bound states. The data from Fenn and Haege quoted permit only an approximate evaluation of the magnitude of the magnesium fractions indicated because few experiments were performed (2 experiments at each magnesium concentration in the medium).

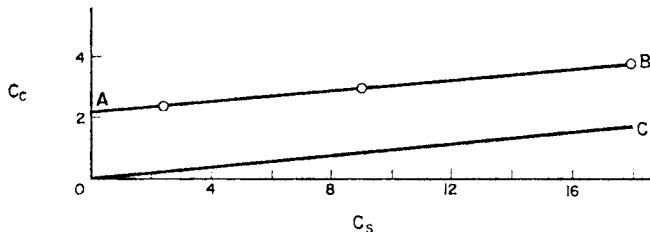


FIG. 121. The dependence of the phosphorus concentration in muscle fibres ( $C_c$ ) on its concentration in the surrounding medium (from Pisareva, 1959)  
 $C_c$  and  $C_s$ —in mg per cent. Temperature 2°. For explanation see text.

The distribution of phosphorus between cells and their bathing fluid in general recalls the distribution of potassium and certain other cations. This was convincingly demonstrated by Briner, Simon and Shaw (1958) and Pisareva (1959, 1961).

Thus, Pisareva (1959 b, 1961) immersed isolated frog sartorius muscles for 8 hr in saline solutions cooled to 2° which contained different concentrations of phosphate with an admixture of radioactive phosphorus. The results of these experiments are shown in Fig. 121. The line AB in this figure reflects the dependence of the concentration of the displaced phosphorus of the muscles ( $C_c$ ) on the concentration of orthophosphate in the surrounding medium ( $C_s$ ). It corresponds to the formula  $C_c = C_s K + A$ . The constant  $A_{\infty}$  in the present case is 2.2 mg per cent. This is the limiting amount of bound phosphorus of the muscles which can be displaced by labelled phosphorus of the medium in these experimental conditions. The coefficient of distribution (solubility)  $K$  is 0.08, that is, independent of the concentration in the medium, its concentration in the water of the muscle fibre in conditions of diffusion equilibrium is 12.5 times lower than in the surrounding solution.

Thus, there are grounds for supposing that the concentration of free inorganic phosphorus in the protoplasm of cells is lower than the concentration in the medium surrounding the cell (the blood plasma). The lower concentration of orthophosphate in the cell is due, evidently, to the lower solubility of ions in the protoplasm as compared with their solubility in the medium, just as happens in the case of ions of potassium, sodium, lithium, chloride and many non-electrolytes. The greater part of the phosphorus present in the cell comes out to form part of organic phosphorus compounds and can be bound adsorptionally or chemically by the cell proteins.

The facts and conclusions presented in this section of the chapter can be reinforced with the data of other workers, especially by those quoted in their papers by Simon, Shaw and others; these workers studied the mechanism of the distribution of ions between sartorius muscles of the toad *Bufo marinus* and solutions of various salts (Shaw and Simon, 1955a, b; Simon *et al.*, 1957, 1959; Briner *et al.*, 1958; Tasker *et al.*, 1959; Frater *et al.*, 1959; Simon, 1959, 1961). These interesting papers will be discussed below.

If the mechanism expounded above of the distribution of inorganic ions between cells and medium happens in reality, then we should conclude that the widely held opinion that ions can diffuse into and out of cells against the concentration gradient is incorrect. The diffusion of a mineral substance in the system cell–medium evidently takes place always in the direction where the concentration of free ions is less than that which should be established on their distribution between protoplasm and medium, as between two phases in conditions of equilibrium.

The diffusion of potassium into a cell against the concentration gradient occurs because it is bound in the protoplasm whereas there is less of it in the dissolved state in the protoplasm than in the medium. Exactly in the same way the diffusion of sodium ions out of muscles after excitation takes place not against the concentration gradient, but along the gradient of the latter, because the equilibrium concentration of this ion in the protoplasm is several times less than in the medium. The same can obviously be said of the diffusion of other ions too and of many non-electrolytes as well.

On the basis of what we have said it is easy to explain all the facts which are frequently cited as proofs of the existence of the sodium pump in the cell membrane. In a series of papers by Conway and his colleagues, and also by other workers, it was shown that if frog muscles are placed for a prolonged period in Ringer's solution with an elevated sodium ion content and free of potassium ions, then the sodium concentration in the muscles grows. When such muscles are transferred to Ringer's solution with a normal sodium ion content and elevated potassium ion content, the sodium ions are observed to emerge from the muscle against the concentration gradient. A similar effect occurs if isolated muscles are placed immediately in Ringer's solution with a lowered sodium content (Steinbach, 1951, 1961; Desmedt, 1953; Shaw and Simon, 1955a; Conway and Carey, 1956; Conway, 1957, 1960a, b;

Carey, Conway and Kernan, 1959; Conway, Kernan and Zadunaisky, 1961; Conway and Mullaney, 1961 etc.).

From our point of view the release of sodium ions from muscles in these conditions is due to the fact that there is direct proportionality between the concentration of sodium ions in the cell and their concentration in the equilibrated medium, the coefficient of proportionality being considerably less than unity. These facts can be explained without the need for recourse to membrane pumps to drive sodium ions out of muscle or any other tissue placed in analogous conditions. The action of enzymatic poisons and other unfavourable factors affecting the sodium ion level in muscle and its rate of release from muscle is explained, in our opinion, by the action of these factors on the properties of the protoplasm as a whole.

Thus, on the basis of the evidence cited, it may be concluded that the distribution of inorganic ions depends on the sorptional activity of the protoplasm and on the change of this activity in shifts of the functional state of the live matter as a whole.

#### *4. On the Nature of the Selective Accumulation of Potassium in Cells*

From our review of the data quoted in this chapter it can be seen that the problem of the selective accumulation of potassium in cells presents itself equally acutely to the membrane and sorptional theories of cell permeability alike. For both theories the solution of this problem depends on finding the differences in the interaction of cations (especially between sodium and potassium cations) with the ionised groups of macromolecules making up the protoplasm.

In discussions of the causes of the asymmetric distribution of potassium and sodium between cells and medium arguments have been adduced to show that potassium, as opposed to sodium, is present in cells principally in the "bound state", but the question of the nature of this "bound state" has not been considered. This question will be discussed below.

The predominance in resting and undamaged cells of potassium, and of sodium ions in the medium, is in general explained by the fact that potassium ions have greater adsorptional affinity to the macromolecules of the protoplasm than sodium ions, as occurs, for example, in soils.

This difference in "affinity" between potassium and sodium was explained by some workers by the different degrees of hydration of these cations (Ratner, 1935, 1950; Epshteyn, 1950, 1957 and others). It was thought that the more strongly hydrated the ion, the more weakly it is adsorbed and the more difficulty it has in forming a chemical bond with anions, and the weaker any such bond will be. In view of this the weakly hydrated potassium ions will compete successfully in the process of ion distribution with the sodium ions, which have a powerful hydrate shell. It is known that the

"energy of absorption" of cations by soil colloids in a series of alkaline metals increases with growing atomic weight of the cations. Their degree of hydration falls off in the same sequence (Gedroits, 1933).

Ling (1952) suggested the hypothesis of fixed charges, which was made to explain the causes of the selective accumulation in cells of potassium rather than sodium. According to this hypothesis, the molecules of the protoplasmic proteins form a three-dimensional network, on whose crosspieces are fixed charges presented by the terminal groups of amino-acid residues. Thus, there is in this system a high density of charges fixed in three dimensions. Ling's hypothesis is also based on the fact that association between oppositely charged ions increases if one of the types of ions is fixed in this three-dimensional lattice.

According to Ling's hypothesis, the selective accumulation of a cation depends on the difference between the electrostatic energy of the fixed negative charge and the cations interacting with it. The energy of the electrostatic bond between oppositely charged ions was determined by the mean dielectric constant of the hydrate shell of the interacting ions and "the distance of least separation" between the fixed anion and the compensating cation. If the least separation is determined by the radius of the hydrated ion, then it follows that potassium ions, being less hydrated, will have greater powers of accumulation than sodium ions.

Ling was soon compelled to make fundamental changes in his hypothesis. The trouble was that the intensity of adsorption in a number of ions of the alkali metals often follows in a different order from that in Hofmeister's lyotropic series. In Hofmeister's series breakdowns are often observed in the sequence of arrangement of cations going as far as complete reversal of the series (see Höber, 1945).

This "rearrangement" in the cation series was explained by the different degree of dehydration of cations in the process of adsorption (Wiegner and Jenny, 1927; Jenny, 1932, etc.). On the basis of similar facts Mullins (1956, 1959a, b) made more precise the theory of the structure of the porous protoplasmic membrane for explaining certain features of the rates of penetration of cations into cells. Bungenberg de Jong (1949, p. 289) was the first to notice that the variations in the polarisation of an anion determines the changes in the arrangement of cations in the lyotropic series. Thus, sulphate colloids fix cations in the order  $K^+ > Na^+ > Li^+$ , and phosphate colloids in the reverse order:  $Li^+ > Na^+ > K^+$ .

The most complete theory of this phenomenon has been developed quite recently by Eisenman and his colleagues (Eisenman, Rudin and Casby, 1957a; Rudin and Eisenman, 1959; Eisenman, 1961). They showed that the order of selectivity in a series of cations is determined by the "strength of the anion field" with which the cations interact, and that with increasing strength of the negative electric field the cation which should be preferentially desolvated is that which is least strongly hydrated.

Eisenman (1961) concluded his article by pointing out that all systems with a very high strength anion field ( $r$  less than  $1.3 \text{ \AA}$ , where  $r$  is the radius of the ionised atom of the ion—the reciprocal of the strength of the ion field) stand out for their high selectivity for hydrogen ions in respect of ion exchange and potential. Systems with lower field strength (with greater values of  $r$ ) are selective for cations of the alkali metals, whose series order will be governed by the variations in the strength of the anion field, so that a system with very low field strength will be selective for potassium, one with intermediate strength for sodium. Further, the writer notes the important part played by the carboxyl and phosphate groups of cell macromolecules in the regulation of the permeability of cells for cations and polar molecules. These groups have anion fields of differing strengths and can ensure the selective adsorption of cations and polar molecules.

Taking into account the fact that the variations in the force of the anion field govern the special order in the selectivity of cations, Ling (1957, 1958, 1960, 1961) put forward a new hypothesis under the name of the "hypothesis of fixed charges" (fixed charge—induction hypothesis). In the new model for calculating the energy of adsorption of various cations account was taken of all the known physical forces (Coulomb, van der Waal's etc.) that determine the interaction of ions and water molecules. Due attention was paid to the fact that the strength of the field of any anion does not always remain constant. This strength, determined as the "effective charge of the anion" (denoted by Ling by the quantity  $c$  and measured in units of length— $c \text{ \AA}$ ), can change in response to changes in a protein molecule at some distance from the ionised group under consideration (induction effect). Such changes in the quantity  $c$  can occur thanks to the appearance of new ionised groups with a protein molecule, thanks to adsorption or chemical binding with a protein of hormones, vitamins, ATP and other active substances and for many other reasons. As a result of these changes in the effectiveness of the anion charge, for example, of a carboxyl group, its adsorptional affinity to potassium can change to a relative affinity for sodium and vice versa.

Ling's hypothesis was invoked to explain such phenomena as the adsorptional selectivity of ion-exchange resins, the accumulation by cells of potassium, the elevation of the permeability of cells for ions of sodium upon excitation and so on.

Against the first variant of the hypothesis of fixed charges a number of objections were raised by Conway (1957). In particular, he estimated that, if Ling's hypothesis is accepted, there is a total of 5–6 per cent of water in the protoplasm that is not available for the dissolving of sodium and chloride ions (water with a low dielectric constant of the solvate shells of ionised groups). If there is no special barrier or pump, as Ling supposes, then the remainder of the water (94 per cent) will be penetrable by sodium and chloride ions without hindrance; thus it is impossible to explain from Ling's position

the very low concentration in the protoplasm of sodium and chloride ions by comparison with their high concentration in the medium.

At this point, however, Conway's criticism does not seem to be very convincing—in building up his theory Ling started from the Donnan principle of ion distribution between cell and medium. In fact, for the structure of the protoplasm suggested by Ling cells cannot have a membrane that is selectively permeable for certain ions or any special pumps. However, even in its last variant Ling's hypothesis cannot be considered to be completely satisfactory, because it does not envisage any special properties for the protoplasm water such as are indicated by the characteristic laws of the distribution between cells and medium of non-electrolytes and a number of other substances in the preceding chapters.

In recent years the problem of the distribution of salts between cells and medium has been the subject of an intensive study by a group of Australian physiologists (Simon, Shaw *et al.*). On the basis of their own investigations and published information they came to the conclusion that the theory of the selective permeability of the cell membrane should be replaced by a theory of the selective adsorption of ions by the macromolecules of the protoplasm (Shaw and Simon, 1955b). Later they proposed the hypothesis of three phases to explain the distribution of ions between muscle and medium (Simon *et al.*, 1957, 1959; Simon, 1959, 1961; Briner *et al.*, 1958, 1959; Frater *et al.*, 1959; Tasker *et al.*, 1959).

One of these phases is the extra-cellular space of muscle, the other two belong to the muscle fibres: these are the "free phase" and the "organised phase" of a cell. The free phase occupies about one third of the total volume of a cell. Ions penetrating a cell are freely soluble in this phase and their concentration in it in the state of equilibrium is equal to their concentration externally.

Those ions which are accumulated by a cell (potassium, magnesium, calcium, inorganic phosphate and so on) are selectively adsorbed by the ultra-structure of the organised phase. This phase is not available to other ions, such as ions of sodium, chloride, lithium, sulphate, bromide etc., or they are excluded from this phase by active transport mechanisms similar to those whose existence in the protoplasmic membrane is postulated. The organised phase embraces various morphological formations: the contracting apparatus, mitochondria, cell nuclei and so forth (see Simon, 1959).

One of the obvious inadequacies of the three phase theory is that cell nuclei and mitochondria do not have the properties of an "organised phase". Thus, it has been demonstrated in Mirsky's laboratory that the sodium concentration in the caryoplasm is many times higher than in the cytoplasm (Allfrey and Mirsky, 1960; Allfrey, Mendt, Hopkins and Mirsky, 1961). The same result is also obtained by other workers (Abelson and Duryee, 1949). Mitochondria, which are also referred to the organised phase, contain a considerable amount of sodium and other ions which should not occur in

this phase (see Harris, 1960). The free intracellular phase is also to some extent "organised": its volume for different ions excluded from the organised phase is different (Simon *et al.*, 1959; Simon, 1959). Consequently, there still remains some doubt about which parts of the cell are to be referred to the "organised phase" and which to the "free phase".

In view of the fact that the individual components when isolated from a cell (nucleic acids, proteins etc.) do not themselves have any noticeable selective adsorption (affinity) in relation to potassium or sodium, some writers have invoked ion-exchange resins as a model for explaining the accumulation of potassium in cells: some of these resins do in fact have a high degree of selectivity for potassium ions (Epshteyn, 1957; Kurella, 1960, 1961). It is considered that the protoplasm is a highly organised polyelectrolytic gel having the properties of an ion-exchange resin with high selectivity for potassium.

It has been established by a number of workers that ions caught by the polyelectrolytic phase of ionite are, in general, in an electrochemically active state and that they have the same properties as ions in an aqueous solution (Katchalsky, 1954; Eisenberg and Fuoss, 1954; Woermann *et al.*, 1956; Rice and Harris, 1956; Hechter *et al.*, 1959; Kurella, 1960, 1961).

This kind of structure and physico-chemical properties of the protoplasm, ensuring the selective adsorption of potassium by cells, is envisaged by many writers.

It should, however, be noted that the electrochemical activity of the potassium ions accumulated by cells can vary within very wide limits, right up to complete loss of activity by at least part of the accumulated ions. This can depend on many features of the active places of the polyelectrolyte where the potassium ions are adsorbed. Experiments with many polyelectrolytes, including protein solutions, convince us of this possibility (Vol'kenshtein, 1947; Bungenberg de Jong, 1949; Ungar and Romano, 1959; Ungar, 1959, 1961). But even this most "firm bond" of potassium should be considered as temporary: in the course of cellular metabolism it may be broken down and recreated, so that even this "bound" potassium of the cell can be exchanged for potassium of the medium, and in certain conditions for other ions as well (Simon, 1959, 1961; Troshin, 1953a, 1960a, 1961; Epshteyn, 1957).

It follows from the material reviewed in this section that there are weighty grounds for looking for the reasons for the selective accumulation of potassium by cells in the laws of ion adsorption by the polyelectrolytic structures of the protoplasm as a whole. Furthermore, it is essential to take into consideration the fact that the water of the protoplasm is probably in a qualitatively different state from the water of the surrounding medium. The fact that different substances differ in their solubility in the protoplasm and are considerably less soluble than in ordinary water can be used as proof of this. Similar views on the properties of the protoplasm and the state of the

water in it (or views close to these) have been expressed at various times in published scientific work (Nasonov and Aleksandrov, 1943; Troshin, 1953a, b, 1959, 1960a, b, c; Ungar, 1959; Tummerman, 1960; Dervichian, 1955; Segal, 1958; Szent-Györgyi, 1957; Baird *et al.*, 1957; Bozler and Lavine, 1958; Bozler, Calvin and Watson, 1958; Bozler, 1959, 1961a, b etc.).

### 5. Conclusions

1. Mineral substances in cells are present in two qualitatively different forms: one part is bound with the colloids of the live matter, the other is dissolved in the protoplasm.

2. The greater part of the total potassium, magnesium, calcium and phosphorus in a cell is present in the protoplasm in the bound state and is incapable of free diffusion. The concentration in a cell of free (dissolved) ions of potassium, magnesium, calcium, sodium, chloride and inorganic phosphorus is several times smaller than in the surrounding medium.

3. The lowered concentration of dissolved inorganic ions in a cell by comparison with their concentration in the surrounding medium is evidence that the protoplasm as a solvent has different properties from the surrounding aqueous solution.

4. It is just these properties of the protoplasm that explain why, in spite of the ability of sodium and chloride ions to penetrate within a cell, their concentration in the protoplasm always remains several times less than in the surrounding medium. The predominance of the concentration of potassium, magnesium, calcium and phosphorus in a cell is achieved thanks to that fraction of these minerals that is bound by the colloids of the live matter.

5. Upon excitation and on damage of the protoplasm there is observed to be a release of potassium, magnesium and phosphate ions from the cell and a movement of sodium and chloride ions in the opposite direction. When the protoplasm changes to the resting state, and also when it returns to normal from a damaged state, on the other hand, potassium and magnesium ions again enter the cell, and sodium and chloride ions are released.

6. The redistribution of mineral ions between cells and medium upon excitation and damage shows that at such times the mineral substances in the protoplasm are liberated from the bound state and change into a form capable of diffusion. The solubility of the ions in the protoplasm is hereupon increased. This is evidently the explanation of the movement of potassium ions out of a cell and of sodium and chloride into a cell when it is excited and damaged.

7. On repair of the damage and the transition of the cell from excitation to the state of rest, the binding of potassium by the colloids is increased again while the solubility of ions in the protoplasm is, on the contrary, lowered. This leads to the re-entry of potassium into the cell and the release of sodium and chloride from it.

## CHAPTER X

# Metabolism and Cell Permeability

### *1. The Relation between Metabolism and Cell Permeability*

Since the time of Pfeffer and until very recently the membrane theory, which made the passage of substances into a cell dependent on their ability to pass through the cell membrane, considered the latter as the organ which regulated metabolism. And in fact, if carbohydrates, amino acids and many other vitally essential substances are not capable of passing through the cell membrane, as was asserted by the classical membrane theory, then in the absence of a substrate it is not possible for the relevant enzymatic reactions to take place, and these substances cannot be used by the cell until the permeability of the membrane has been somehow changed. The effect of metabolism on cell permeability is acknowledged insofar as intracellular enzymatic reactions can lead to a change in the semi-permeable properties of the membrane, and in as much as these reactions result in the formation in the cell of substances which require removal from the cell and are capable of being exchanged for other substances in the surrounding medium.

From the point of view of modern membrane theory, the permeability of a cell for a number of substances is seen as an active process in the sense that the passage of substances into a cell and their exit therefrom, as well as their accumulation in the cell, are controlled by enzymes localised in the membrane, and in that the passage of substances into the cell and the asymmetric distribution of them between cells and medium are thought of as active processes taking place with expenditure of a definite amount of metabolic energy (Krogh, 1946; Wilbrandt, 1947, 1961; Rosenberg, 1948; Kamen and Spiegelman, 1948; Sacks, 1948; Ussing, 1949, 1953; Brown, 1952; Rosenberg and Wilbrandt, 1952; Rothstein, 1954a, b, c, 1959, 1961; LeFevre, 1955; Park, 1961; Morgan, Post and Park, 1961; Randle, 1961; Quastel, 1961; Mitchell, 1961 etc.).

According to the new membrane theory, the penetration into a cell of substances in most cases follows the scheme; substance A, dissolved in the medium, is converted in the cell membrane into substance B and in this form passes into the cell; in the cell this substance is reconverted into substance A (Ussing, 1949). For example, sugars, glycerol and the anion of phosphoric acid before getting into the cell are first taken up in the membrane into the composition of phosphoric esters (hexose phosphates, glycero-

phosphates, etc.) which are capable of penetrating the cell and, upon penetration, take further part in cellular metabolism.

Rosenberg and Wilbrandt (1952) point out that the former concept of the cell membrane as a passive formation must be completely abandoned: it must be seen as part of the cell, controlling with the aid of enzymes the process of cellular permeability. The same point of view on this problem is expressed by Kamen (1949). He writes that the permeability of a cell is not simply controlled by the laws of diffusion, but is affected principally by cellular metabolism. The accumulation of any mineral substance in a cell is caused, so this author supposes, not by any physico-chemical selective permeability effect based on membrane potentials, but by specific chemical processes in the cell which control the action of the enzymes.

It can be seen from these statements that this concept of cell permeability is essentially a development of the idea of Overton (1895) of the "adenoidal activity" of cells and of the concept expressed by Höber as early as 1905 of "physiological" or "active" permeability as opposed to the purely "physical permeability of cells".

"Physiological permeability" is taken by Höber (1945), as is well known, to cover all those cases when the passage of a substance into or out of a cell is accomplished against the concentration gradient and when its distribution between cell and medium cannot be explained by Donnan equilibrium or chemical and adsorptional binding by the protoplasm colloids.

However, no satisfactory explanation has been offered to date of the unsymmetrical distribution of substances between cell and medium and their redistribution upon excitation of and damage to the cells. In explaining these phenomena within the framework of the membrane theory writers have had to resort to postulating the existence in the cell of mechanisms for the "expelling" (Dean, 1941a; Krogh, 1946; Ussing, 1949) or continuously "pumping out" (Hodgkin, 1951, 1958; Conway, 1951, 1953, 1955, 1957, 1959, 1960a, b) of the cell of some substances and of the "active transport" into the cell of others.

The necessity of postulating the existence in cells of such mechanisms drops out if account is taken of the facts considered in the preceding chapters which indicate that the solubility of substances in the protoplasm is considerably lower than their solubility in the aqueous surrounding medium and that the substances which penetrate cells are bound, some more, some less, by the colloids of the protoplasm. This is recognised by the adherents of the membrane theory themselves. Thus Ussing (1949), citing data from a number of writers which point to the special affinity of myosin to potassium, came to the conclusion that it is difficult to speak of the active transport of potassium into a muscle. He wrote: "In fact, there is little basis for assuming the existence of active transport of potassium into the fibres" (p. 134).

The sorptional theory of cell permeability also starts from the fact that the absorption of substances from the medium by a cell depends on the enzy-

matic activity of that cell. However, it considers to be utterly unnecessary the assumption that there are localised in the cell membrane enzymes which regulate the permeability and distribution of substances between cells and medium.

In fact, in a number of cases the rate of passage of substances into a cell and their distribution between the cell and the medium are directly dependent on the rate of metabolism: the faster these enzymatic reactions take place which result in the conversion of penetrating substances into other chemical compounds which go to make up and renew the cell structures or are used as energy sources, the greater will be the permeability of the cell for these substances. But this can occur only in those cases where there is in the surrounding medium a sufficiently high concentration of the substrate of the enzymatic reactions. When this is not so, a mechanism in the cell comes into action which ensures an adequate flow of substances from the surrounding medium, thanks to which the enzymes are enabled to work at their optimum level. This can be ensured, as we have seen, by the adsorptional and chemical binding of substances by the colloids of the cell structures. It is just in this, so it seems to us, that is revealed the close connection and inter-dependence of metabolism and cell permeability.

Besides the indicated relation, metabolism is the factor which creates in a cell the conditions which cause increase or decrease in the excretion of those substances which do not take direct part in enzymatic reactions; this can happen because the enzymatic reactions result in the chemical and adsorptional activity of the cell colloids in respect of these substances being raised or lowered respectively. This is indicated by the facts reviewed in our discussion of the permeability of cells for non-electrolytes, vital dyes, inorganic substances etc.

As has already been stated, there are no direct proofs that the enzymes which control the passage of substances into a cell are localised on its surface (Rosenberg and Wilbrandt, 1952).

As proof of the existence in the cell membrane of any cell of a special mechanism (enzyme systems, carriers) for regulating cell permeability and selective and active transport, many scientists cite the activity of the cells of the secretory and resorbing epithelia lining the inside of the renal tubules, many glands, the mucous membrane of the stomach and intestine and so on (Rosenberg and Wilbrandt, 1952; Conway and Hingerty, 1953; Widdas, 1954a; LeFevre, 1955 and others). This line of argument seems to me to be unconvincing.

The cells named are highly specialised. Their special function in the organism consists in the selective displacement (pumping over) of solutions of various substances in the basal or apical direction, often against the concentration gradient. Although, as we have already seen, secreting and resorbing cells do not differ in any way from other cells in respect of their permeability, nonetheless it is quite clear that to achieve their function of secretion and

resorption there must exist some mechanism or other working at the expense of metabolic energy. The morphology of these cells shows clearly that such a "pump" mechanism is to be found inside, and not on the surface of, the cells.

All cells of the resorbing and secreting epithelia are morphologically characterised, first and foremost, by the polarity and asymmetry of their structure. The mitochondria in these cells, as a rule, are substantially concentrated in the basal or apical part. Golgi's apparatus is to be found near any one pole. Histochemistry has shown that many enzyme systems, for example, phosphatases, are disposed polarly in such cells. These features of the structure of the cells named are indubitably to be connected with their specific function.

The mechanism for transmitting substances by secreting and resorbing cells can be represented in the following way. Let us suppose that substance A is pumped away by the cells in the basal direction, as happens for glucose, sodium ions and possibly other substances in the renal tubules. Substance A, which passes into the apical part of the cell along the concentration gradient, is here converted into a complex compound AB of high molecular weight or colloidal nature, B being the acceptor, the carrier of the substance A. This compound AB can move freely within the limits of the cell; in its basal part it can be split afresh into the substances A and B. The concentration of the dissolved (free) substance A in the basal part can reach any value. This should depend exclusively on the efficiency of the mechanism in the apical part of the cell which converts the substance A into the compound AB, and also on the efficiency of the mechanism which splits this complex AB in the basal part of the cell into A and B. As a result of this the conditions are created for the exit of substance A from the cell in the basal direction along the concentration gradient. This will also be assisted by the fact that, as we have seen, the solubility of substances in the protoplasm is considerably lower than in the surrounding medium. This will prevent the free diffusion of the substance A across the layer of epithelial cells.

The hypothesis suggested here is not in principle new. Something of the same kind has been published about the accumulation of inorganic substances in the vacuoles of plant cells (Brooks, 1937) and about the mechanism of the activity of secreting and resorbing cells (Troshin, 1958; Nasonov, 1959a).

## *2. The Temperature Dependence of the Absorption of Substances by Cells*

The close connection between the permeability of cells and metabolism is also clearly revealed in the temperature dependence of the processes. It is well known that with increasing temperature the coefficient  $Q_{10}$  of the rate of absorption of substances by a cell from the medium increases not by 1.5–2.5 times, as would be expected if purely diffusional transport of sub-

stances lay at the basis of cell permeability, but many times more than this, which indicates the more complicated nature of this phenomenon.

Thus, Masing (1914) in experiments with human erythrocytes on the process of the absorption by cells of glucose obtained the following values of the temperature coefficient: from 0 to  $10^{\circ}$ —12, from 5 to  $15^{\circ}$ —6, from 10 to  $20^{\circ}$ —3·5, and from 15 to  $25^{\circ}$ —2·3. Ege (1924) also obtained large values of  $Q_{10}$  (from 2·2 to 3·7) for the penetration of inorganic substances and organic acids into red blood corpuscles. Gellhorn (1929, pp. 67–9 and 109–112) cites a large volume of evidence obtained in experiments with animal and plant cells which shows that the  $Q_{10}$  of the permeability of cells is considerably higher than the  $Q_{10}$  of diffusion processes. Large values of the temperature coefficient were also obtained by Kolotilova and Engelgardt (1937), Reinwein *et al.*, (1957) and others for the absorption of sugars by rabbit and human erythrocytes.

The magnitude of the temperature coefficient of permeability of the same cells for a number of substances varies within very wide limits. Thus, Jacobs, Glassman and Parpart (1935) determined the mean value of  $Q_{10}$  of the permeability of rabbit erythrocytes for a number of substances. It was found to be as follows:

Water	1·32	Ethyleneglycol	1·65
Glycerol	1·46	Diethyleneglycol	2·69
Thiourea	1·86	Triethyleneglycol	3·55

According to the data of the same authors, the temperature coefficients of permeability of the erythrocytes of different types of mammals for any one substance also differ very strongly from one another. Thus, for example, the  $Q_{10}$  for the rate of penetration of glycerol into mammalian erythrocytes was found to be as follows:

Rat	1·05	Dog	3·42
Human	1·15–1·28	Pig	3·70
Rabbit	1·46	Bull	3·77
Guinea pig	1·63		

These data indicate that high values of  $Q_{10}$  cannot be used as proof of the occurrence of chemical transformations of a substance in the process of its penetration into a cell. On the contrary, we should suppose that water and glycerol penetrate rabbit erythrocytes by a purely diffusional route, while the penetration into these cells of ethyleneglycol and di- and tri-ethylene-glycol and thiourea is attended by their chemical transformation. It is also hardly probable that glycerol penetrates rat, human and rabbit erythrocytes by a diffusional route and dog, pig and bull erythrocytes with the assistance of chemical reactions.

This is shown by other data as well. Thus, Cori and his colleagues have demonstrated in a series of papers that the rate of penetration of a number of

unphosphorylated pentoses and hexoses into the muscle fibres of rat diaphragms is practically or completely independent of temperature in the range 17–37°, whereas the  $Q_{10}$  of the permeability in these conditions of 2-deoxyglucose, glucose and pentoses in the presence of insulin is about 2, and for the utilisation of glucose about 2.5. The  $Q_{10}$  of the rate of penetration of pentoses and hexoses in the temperature range from 0–12° is excessively large (Kipnis and Cori, 1957, 1959; Kipnis, Helmreich and Cori, 1959; Helmreich and Cori, 1957). Similar data are reported in a number of other papers (Krane and Crane, 1959; Burger *et al.*, 1959 and others).

The very high values of the temperature coefficient of the permeability of cells for a number of non-electrolytes (see Table 99) are evidently explained by many causes, especially by physical changes of the whole protoplasm and of its superficial layers (see Rubinshtein, 1947). However, in a number of cases when non-electrolytes are utilised by cells, the value of  $Q_{10}$  is undoubtedly affected also by the chemical transformations of these substances in the protoplasm.

TABLE 99. THE EFFECT OF TEMPERATURE ON THE PERMEABILITY OF CELLS FOR VARIOUS SUBSTANCES

Types of cell, substances	Temperature zone	$Q_{10}$	Author
Yeast cells, <i>S. cerevisiae</i> , entry of <i>d</i> -galactose	1–23°	2.9	
exit of <i>d</i> -xylose	1–23°	2.4	Burger, Hejmova and Kleinzeller, 1959
Ehrlich ascites cells, permeability for:			
methylglucose	20–30°	4.0	Crane, Field and Cori, 1957
ribose	20–30°	3.8	
Rat diaphragm muscle fibres			
permeability for <i>d</i> -xylose			
in the presence of insulin	27–37°	1.75	Kipnis and Cori, 1957
Leaf cells of			
<i>Majanthemum bifolium</i> ,			
<i>Convallaria majalis</i> and			
<i>Salvinia natans</i>			
Permeability for:			
water	22–36°	1.53	Seemann, 1950b
urea	22–36°	2.25	
		—2.47	

Hahn and Hevesy (1942) studied the permeability of rabbit erythrocytes for radioactive phosphorus and found that at 37° considerably more phosphate penetrates the erythrocytes than at 0°. As the temperature is raised from 0° to 37° the rate of penetration of the phosphates into the blood corpuscles increases 10–12 times.

According to Eisenman *et al.* (1940) labelled phosphate is absorbed by human erythrocytes 30 times faster at 38° than at 7°.

The labelled phosphate which passes from the plasma or artificial saline medium into erythrocytes, just like the unlabelled, participates in the exchange with all the phosphorus-containing organic compounds. It has been established that these compounds differ from one another very strongly in the rate of inclusion into them of labelled phosphorus. Phosphate is most rapidly included into the molecules of the phosphoric esters (adenosinetriphosphate, hexose phosphate, phosphopyruvate, etc.). The exchange of the phosphorus groups of the lipids takes place very slowly (Hevesy and Hahn, 1940; Hahn and Hevesy, 1942; Hevesy, 1951, and others).

A detailed study of the temperature dependence of the phosphorus exchange of human erythrocytes has been made by Vladimirov, Pelishchenko and Urinson (1947) and Vladimirov, Ashmarin and Urinson (1953). They found that at body temperature a considerable amount of labelled  $^{32}\text{P}$  of the inorganic phosphate penetrates the cells in exchange for intracellular inorganic phosphate. In this process there is rapid exchange of the labile phosphate of adenosine-phosphoric acid and the phosphate of phosphoglyceric acid, whereas the exchange of lipid phosphorus occurs only at a very slow rate. Lowering the temperature has the effect of greatly depressing these exchange reactions.

Vladimirov, Ivanova, Pravdina and Rubel' (1959) showed that in strong hypothermy (18–20°) in the case of rats there is observed to be a sharp lowering in the rate of movement of radioactive phosphorus into the brain from the blood and an increase in the specific activity of the inorganic phosphorus in the blood and brain. In these conditions the rate of renewal of the organic phosphorus compounds in the brain, especially of the lipids, drops sharply.

If we take into account the fact that, as we have seen, the great mass of cell phosphorus is incorporated in various organic phosphorus compounds, while there is only an insignificant fraction of free phosphate anion, it becomes clear that the rate of penetration of this ion into a cell and its exchange for intracellular phosphate will depend almost entirely on the synthesis and renewal of the organic compounds in which it is incorporated. Since the activity of the enzymes responsible for the synthesis and renewal of organic phosphorus compounds is almost entirely halted at temperatures close to zero and grows strongly with increase in temperature, we accordingly find that high values of  $Q_{10}$  of the permeability of cells are found for this anion.

The same could also be said in respect of the absorption by cells of amino acids, sugars and many other substances. The relevant material in proof of this is to be found substantially in the reviews of Borsook (1950), Hevesy (1951) and others.

If the concentration in the surrounding medium of phosphate, potassium, sugars, amino acids and other substances is increased to many times greater

than normal, the amount absorbed by the cell increases only slightly; also the rate of absorption by the cells increases many times less with increasing temperature than it does with increasing temperature in physiological or lower concentrations of these substances in the medium (Eisenman *et al.*, 1940; Hahn and Hevesy, 1942; Borsook, 1950, etc.).

In a number of cases high temperature coefficients are obtained for the exchange of potassium, sodium and other cations, and also for water molecules or for the loss of these substances by cells (Kalman, 1959; Klein, 1959; Humphrey, 1959; Humphrey and Johnson, 1960 etc.). Thus, in a study of the rate of exchange of sodium of frog muscle for sodium of Ringer's solution

TABLE 100. THE EFFECT OF TEMPERATURE ON THE POTASSIUM AND SODIUM ION FLUX INTO HUMAN ERYTHROCYTES (from Glynn, 1957a)

Flux	Temperature zone, °C	Apparent activation energy, kcal/mole	Author
Total potassium ion flux inwards	27-37°	14.5 ± 1.3	Raker <i>et al.</i> , 1950
Total potassium ion flux inwards	-	15.8	Sheppard and Martin, 1950
Total potassium ion flux inwards	-	16.2	Ponder, 1950
Total potassium ion flux inwards	34-39°	12.2 ± 1.4	Solomon, 1952a
Linear component of potassium flux inwards	29-37.7°	15.0 ± 2.2	Glynn, 1956
Michaelis component	29-37.7°	14.1 ± 0.9	Glynn, 1956
Potassium ion flux outwards	34-39°	12.4 ± 1.3	Solomon, 1952a
	-	15.8	Sheppard and Martin, 1950
Total potassium ion flux outwards	32-37°	14.9 ± 3.4	Solomon, 1952a
Total potassium ion flux outwards	24-27°	18.0	Clarkson and Maizels, 1956
Active component of sodium flux outwards, connected with potassium	29.9-37.7°	14.5 ± 0.8	Glynn, 1956
Passive component	29.9-37.7°	15.3 ± 0.6	Glynn, 1956
Total sodium ion flux inwards	32-37°	20.2 ± 2.7	Solomon, 1952a
Total sodium ion flux inwards	24-37°	15.0	Clarkson and Maizels, 1956

both potassium-free and containing 4 mequiv/l of potassium  $Q_{10} = 1.9 \pm \pm 0.1$  (Edwards and Harris, 1957). Very high values of the apparent energy of activation for the processes of cation exchange in human erythrocytes have been obtained by many workers (see Table 100). However, as has been rightly noted by Maizels (1954 a, b), a high energy of activation for ion movement into cells does not give us any right to assume that there are chemical processes taking place at this point.

### 3. *The Effect of Respiration, Metabolic Inhibitors and Other Factors on the Permeability of Cells*

The dependence of the absorption of substances by cells on the intensity of demand for oxygen is best studied in plant cells. This problem is discussed in detail in works by Lundegårdh (1937), Sabinin (1940), Krogh (1946), Ratner (1950) and others. We shall here cite only the principal results from certain of the numerous works on this subject.

Thus, Briggs (1930) observed that the rate of penetration of inorganic ions into plant cells and their accumulation in the cell sap depends on the intensity of respiration. According to his ideas, respiration results in the formation in the protoplasm of hydrogen cations and carbonic acid anions which are exchanged through the cell membrane for cations and anions of the surrounding solution. The greater the formation of  $H^+$  and  $HCO_3^-$  in the protoplasm, the more strongly marked will be the absorption of inorganic ions from the medium.

Lundegårdh and Burström (1933a, b; also Lundegårdh, 1935) put forward the well-known theory of "anion respiration", according to which the passage of anions of the surrounding medium into a cell and the intensity of respiration are interdependent. From the results of other workers (Steward, 1932a, b, c, 1933, 1935, 1937; Steward *et al.*, 1932, 1936; Jenny and Cowan, 1933; Hoagland and Broyer, 1936; Hoagland, 1940, 1944; Sutcliffe, 1954, and others) and later researches by Lundegårdh (1937, 1938, 1940, 1945, 1954, 1955) it became clear that the rate of absorption by a cell of both anions and cations depends on the intensity of respiration. Lundegårdh and Burström (1944/5) showed that the intensity of respiration is to a considerable extent determined by the accumulation of carbohydrates.

Hoagland and Broyer (1936) discovered that the roots of aqueous barley cultures, when placed in a potassium bromide solution with air blown through the solution for 6 hr, increase their potassium concentration from 29.7 to 61.3 mequiv/l of cell sap and their bromide concentration from 0 to 26 mequiv. If the potassium bromide solution with the barley roots in it has nitrogen blown through for 6 hr, almost all the supplementary quantity of potassium and bromide absorbed leaves the cells; when air is once again blown through these substances are found in about the same quantities in the cell sap. The authors conclude from this experiment that the passage of inorganic

ions into a cell and their accumulation in the cell sap are maintained at the expense of respiratory energy.

Hoagland (1940), in a discussion of his experimental data and the work of other authors, came to the conclusion that the protoplasm of plant cells is permeable for cations as well as anions. The preferential accumulation in the cell and greater rate of penetration of some ions compared with others through the cell membrane is connected by him with the biochemical reactions which take place in the cell in aerobic conditions: with the synthesis of proteins, organic acids, esters, with the utilisation of carbohydrates, etc.

The established fact that the passage of substances into a cell depends on the intensity of respiration gave rise to a large amount of work devoted to the effect of various metabolic inhibitors on the permeability of animal and plant cells.

Thus, Kramer (1951) in experiments with roots of *Pinus taeda* studied the effect of respiratory inhibitors (sodium azide  $-0.001\text{ M}$ , sodium fluoride  $-0.005\text{ M}$  and malic acid  $-0.025\text{ M/l}$ ) on the absorption by cells of radioactive phosphorus at various pH's. The sodium azide reduced the accumulation of  $^{32}\text{P}$  in the cells at all pH values, the sodium fluoride depressed it at low pH values and the malic acid lowered it at low values but, on the other hand, raised the accumulation of  $^{32}\text{P}$  at high pH values.

Hahn and Hevesy (1942), studying phosphorus exchange in rabbit erythrocytes, showed that the addition to the blood of potassium cyanide depressed the rate of exchange of phosphorus by approximately 50 per cent. The labelled phosphate passing into the cells was for the greater part found in the inorganic fraction. In the opinion of the writers, this indicates that the presence of cyanide affects the rate of exchange of the phosphate of the medium for erythrocyte phosphate not by changing the permeability of the erythrocyte envelope, but by inhibiting the process of the renewal of the organic phosphorus compounds.

Rapoport, Leva and Guest (1941) found that potassium cyanide prevents the inclusion of radioactive phosphorus in the phytic acid and lipids of goose erythrocytes. The placing of the erythrocytes in an atmosphere of nitrogen causes a similar effect. According to Mueller and Hastings (1951), the addition of fluoride to blood leads to a sharp increase in the amount of inorganic phosphate of the plasma and to a decrease in the ATP content of the erythrocytes. Monoiodoacetate caused an increase in the inorganic phosphate content of the plasma and erythrocytes at the expense of the ATP and the diphosphoglyceric acid. Kaplan, Memelsdorff and Dodge (1945) showed that slices of rat kidney and liver, when incubated in a medium containing radioactive phosphate in the absence of oxygen, have considerably depressed inclusion of  $^{32}\text{P}$  in the organic phosphorus compounds.

Mullins (1954), in experiments with sciatic nerve and Pisareva (1959b) in experiments with frog muscles, established that the absorption of radioactive phosphorus from the surrounding saline medium is depressed by the

absence of oxygen, by lowering of the temperature, low azide concentrations and increased calcium ion concentration. Stimulation of the nerve depresses the absorption of labelled phosphorus but does not affect the amount of  $^{32}\text{P}$  absorbed earlier by the nerve.

According to Kamen (1949) oxidising inhibitors, such as azide and cyanide, depress lipid synthesis by destruction of anaerobic glycolysis, with which the synthesis of these phosphorus-containing compounds is connected.

However, Vladimirov (1946), using radioactive phosphorus to study the effect of oxygen starvation on phosphorus exchange in various phosphorus fractions of rat liver and brain, established that in the cells of the liver of the experimental animals the exchange of the phosphorus of the acid-soluble fraction of the phosphorus compounds takes place at the same rate as in the liver of the controls. In 8 hr there is observed to be exchange of almost all the molecules of the liver phospholipids both in conditions of hypoxia and in normal conditions. The exchange of the protein phosphorus takes place at a significantly slower rate than the exchange of the lipid phosphorus. The penetration of labelled phosphate from the blood into the brain tissue and the metabolism of the phosphorus compounds of the brain are accomplished considerably more slowly than the metabolism of the phosphorus compounds of the liver. This is evidently due in part to the poor permeability of the barrier between the brain and the blood for phosphate.

According to Smirnov and Chetverikov (1953 a, b) an inadequate supply of oxygen to rabbits leads to accelerated inclusion of labelled phosphate of the blood in the organic compounds of the cortex and medulla oblongata. They attribute this acceleration to the acceleration of the penetration of phosphate of the blood into the brain tissue. These authors also suppose that the acceleration of the exchange (and, consequently, of the penetration) of phosphate in hypoxia occurs at the expense of the acceleration of the exchange of the phosphorus of the lipid fraction in the brain tissue itself.

Besides oxygen starvation, effects on the rate of penetration of phosphate into cells caused by a number of other factors which change the rate of metabolism have been discovered by many workers. Thus, Grodzenskii and Kiseleva (1949) found with the aid of radioactive phosphorus that insufficient protein in the food of growing rats accelerates the penetration and exchange of phosphorus in the liver cells. Esterification of phosphorus is particularly enhanced, then synthesis of phospholipids, but the synthesis of phosphorus-containing proteins is depressed. Further, Guberniyev and Il'yina (1950), on introducing radioactive phosphorus in the form of  $\text{Na}_2\text{HPO}_4$  intravenously into dogs, found that in as little as 40 min there was labelled phosphorus included in the nucleoproteins in the digestive glands. When the secretory activity of the glands was stimulated by the introduction of pilocarpine into the blood, the metabolism of nucleoprotein phosphorus was increased many times. This is intelligible because the excretion of secretory products requires a con-

stant influx into the cell of the initial substances from which they are formed, which will find expression in elevated permeability of the secretory cells for these substances.

Using radioactive phosphorus in experiments on dogs with a fistula of the gall bladder, Grodzenskii, Zamyshkina and Koroleva (1947) discovered that labelled phosphate passes at a high rate from the blood to the hepatic cells and takes part in the formation of organic phosphorus compounds. In as little as 30 min after the intravenous introduction of labelled phosphate, the gall showed noticeable radioactivity. Morton and Schwartz (1953) showed that slices of the thyroid gland, liver and kidneys on incubation in the presence of  $^{32}\text{P}$  are permeable for phosphate and capable of including it in the synthesised lipids. On the addition to the incubation medium of thyrotropine the rate of synthesis of labelled lipids in the thyroid gland slices increases about 2·5 times, but no increase is seen in the case of the liver and kidney sections. This phenomenon will be connected with iodide metabolism in the thyroid gland (the use of inorganic iodide in the synthesis of diiodotyrosine and thyroxine). Hokin and Hokin (1953) established with the aid of radioactive phosphorus that the permeability of cells of slices of the pancreas, liver and brain of dogs and of the liver, ventricles, kidney cortex and cerebral cortex of guinea pigs for phosphate changes considerably depending on the action of various factors. Thus, stimulation of the secretion of enzyme in the slices of pancreas with carbamylcholine or acetylcholine increased the inclusion of  $^{32}\text{P}$  in the phospholipid fraction by 7 times compared with the control. The stimulatory effect of carbamylcholine on secretion and inclusion of  $^{32}\text{P}$  in the phospholipids was reduced by atropine. On incubation of the slices in anaerobic conditions the inclusion of labelled phosphorus in the acid-soluble phosphoric esters was reduced to 34 per cent by comparison with aerobic conditions (see review of Hokin and Hokin, 1961).

Many workers have established that orthophosphate quickly penetrates actively metabolising cells and that phosphorus is accumulated in large amounts in them. If these same cells are deprived of sugar, then the orthophosphate penetrates slowly and the cells can even lose phosphate to the surrounding medium. This was found in experiments with yeast cells (Hevesy *et al.*, 1937; Mullins, 1942b; Spiegelman, Kamen and Sussman, 1948; Rothstein, 1959), with bacteria (Wiggert and Werkman, 1938; O'Kane and Umbreit, 1942; Vogler and Umbreit, 1942, etc.) and with brain cells (Schachner *et al.*, 1942). It was also noticed that in actively metabolising cells the inorganic phosphorus concentration can be lower than in weakly metabolising cells which have a limited amount of substrate in the surrounding medium (Wiggert and Werkman, 1938; O'Kane and Umbreit, 1942; Schachner *et al.* 1942; Lundsgaard, 1938, and others).

Increased absorption by cells of orthophosphate from the medium is connected with the synthesis of organic phosphorus compounds and the

release of phosphates from a cell with their decomposition. This is supported by the data of many writers who have observed an increase in the absorption of orthophosphate after the fertilisation of egg cells (see p. 213).

According to the observations of Friedlander, Perlmen and Chaikoff (1941) the absorption of radioactive phosphorus from the blood stream by denervated muscles in rats is 5 times greater than by normal muscles.

Borsook (1950) in his review article on the inclusion of amino acids added to the medium in the cell proteins as a synthetic process which takes place at the expense of respiratory energy, cites many facts which indicate that anaerobiosis, arsenite, arsenate, azide, dinitrophenol, fluoride, cyanide and other substances depress the utilisation by cells of various labelled amino acids (glycine, leucine, lysine, methionine, alanine etc.) for protein synthesis. Clearly, this leads to lowered permeability of the cells for these substances.

The effect of metabolism on cell permeability is also shown by a review of the distribution of potassium between live matter and the surrounding medium. If the predominant part of the cell potassium (just like the phosphorus) really is bound somehow or other by the colloids and its dissolved part in the protoplasm is insignificant (about half that in the medium), then, when there is an increase in the mass of live matter during growth of the cells while at the same time there is continuous exchange of potassium of the cell for potassium of the medium, the permeability of the cell for this element is determined almost exclusively by the synthesis and change in properties of the organic compounds with which it is bound.

It is well known that when blood is stored in low temperature conditions, a considerable amount of potassium passes from the erythrocytes to the plasma. As the temperature is raised the potassium again returns to the cells and can reach its initial level. During the entry and exit of the potassium from the cell there is, as a rule, diffusion of sodium in the opposite direction. The loss by cells of potassium and the absorption by them of sodium upon cooling is observed in experiments with the erythrocytes of humans and various animals (Wilbrandt, 1938 b, 1947; Danowski, 1941; Harris, 1941; Sheppard and Martin, 1950; Ponder, 1951; Maizels, 1951, 1954 a, b; Solomon, 1952 b; Bernstein, 1953, 1954; Bersin, 1953; Straub, 1953 a, b; Hunter *et al.*, 1956, etc.), with polymorphonuclear leucocytes of rabbits (Hempling, 1953, 1954; Elsbach and Schwartz, 1959), with isolated rabbit eye lens (Harris, Gehrsitz and Nordquist, 1953), with yeast cells (Conway and Hingerty, 1953), with the cells of the sea algae *Ulva lactuca* (Hayward and Scott, 1953), with the muscles of frogs and other animals (Wesson, Cohn and Brues, 1949; Steinbach, 1951, 1961; Stephenson, 1953 a, b, 1957; Carey, Conway and Kernan, 1959; Cosmos and Harris, 1961), with isolated frog nerve (McLennan and Harris, 1954), and with slices of various organs and tissues (Mudge, 1951 a, b; Manery and Husdan, 1956; Whittam and Breuer, 1959; McLean, 1960; Broome and Opie, 1960 etc.).

In all cases when isolated cells are kept in artificial media the presence of glucose in the incubation liquid facilitates the maintenance in the cells of a high potassium concentration and a low sodium concentration. Even in cold conditions the addition of glucose is sufficient to restore the lost ability of the erythrocytes to accumulate potassium (Harris, 1941; Maizels, 1949, 1951; Sheppard and Martin, 1950; Bernstein, 1954, and others). A similar effect is also produced by certain other hexoses (Maizels, 1951). At the expense of aerobic glycolysis nucleated erythrocytes can maintain a high potassium concentration (Maizels, 1954 a, b; Tosteson, 1954; Tosteson and Robertson, 1956).

A number of authors have shown that anoxia leads to the loss by cells of potassium and the entry into them of sodium. This was proved in experiments with frog sciatic nerve (Fenn and Gerschman, 1950; Harreveld, 1950, 1951), with crab nerve (Shanes, 1950), with the giant fibre of the squid (Shanes and Berman, 1955a) and with slices of cortex of the kidney of rabbits, rats, guinea pigs (Mudge, 1951a, b; Whittam and Davies, 1953, 1954; Aebi, 1953; Whittam, 1956, 1960 etc.).

The same effect is produced by all poisons that depress respiration or glycolysis, such as cyanide, azide, dinitrophenol, iodoacetate, fluoride, etc. (Harris, 1941; Conway and O'Malley, 1946; Flink *et al.*, 1950; Maizels, 1951, 1954 a, b, 1956; Mudge 1951 a, b; Aebi, 1951, 1952a, b; Green and Parpart, 1953; Conway and Hingerty, 1953; Hayward and Scott, 1953; Scott and Hayward, 1954; Robinson, 1954; Sutcliffe, 1954; Hurlbut and Brink, 1954; Hurlbut, 1958; Briner *et al.*, 1959; Frazier and Keynes, 1959; Hicklin, 1960; Whittam, 1960 and others, see references in: Shanes, 1958; Epstein, 1959; Rothstein, 1954a, 1960, 1961; LeFevre, 1955).

The rate of efflux of sodium ions of the giant fibre of the squid and cuttlefish falls sharply in the presence of dinitrophenol, azide and cyanide (Hodgkin and Keynes, 1955a, b, 1956). The introduction into the fibre of ATP and argininephosphate restores the former rate of efflux of sodium from the poisoned fibre (Caldwell, Hodgkin, Keynes and Shaw, 1960a, b; Caldwell and Keynes, 1959; Caldwell, 1960a, b; Keynes, 1961).

Straub and Gardos suppose that potassium ions are actually accumulated in human erythrocytes using the energy of decomposition of ATP, which is resynthesised in the course of glycolysis (Straub, 1953a, 1956; Gardos, 1954, 1956, 1958, 1961; Gardos and Straub, 1957).

Schatzmann (1953) showed that cardiac glycosides inhibited the active uptake of potassium and extrusion of sodium from erythrocytes, and Glynn (1956), in extending these studies, suggested there may be competition between the glycoside and potassium ions.

Cardiac glycosides (strophantin, ouabain), like many other poisons too, lead to the redistribution in cells of potassium and sodium (Harris and Prankerd, 1955; Harris, 1957a; Edwards and Harris, 1957; Caldwell and Keynes, 1959; Post, 1959; Caldwell, 1960a, b; Post and Albright, 1961).

In all cases of cell damage, redistribution of electrolytes takes place in the conditions of breakdown of the basic systems of metabolism and in these conditions the cell is incapable of maintaining inside itself an electrolyte composition different from that of the surrounding medium.

When a suspension of the erythrocytes of vertebrates and humans is subjected to haemolysis (methyl, ethyl and butyl alcohol, guaiacol, X-rays and the like) in doses lower than the haemolytic or for periods insufficient for the onset of haemolysis, there is observed to be an increase in the rate of displacement of intracellular potassium by sodium of the medium (Ponder, 1947a, b, 1949, 1951; Parpart and Green, 1951; Sheppard and Beyl, 1951 etc.). After the removal of the haemolytic from the erythrocyte suspension the directions of movement of the potassium and sodium are once again reversed. This reversibility of the exchange of potassium for sodium also occurs when glucose is present in the surrounding medium. Ultraviolet rays also lead to reversible loss of potassium by cells (see references in Green, 1956; Sanders and Giese, 1959).

Cowie, Roberts and Roberts (1949) also showed that the rate of absorption of potassium by the bacteria *Escherichia coli* is greater the greater the amount of glucose in the medium. The rate of absorption of potassium by other cells (yeast cells, protozoa, frog egg cells) also increases with increasing glucose concentration in the medium. These authors assert that the potassium is bound within the cells in the form of a hexosephosphate salt.

Krebs, Eggleston and Terner (1951) found that slices of guinea pig brain cortex, when incubated for a few minutes in a saline medium or homologous serum, lose up to 40 per cent of their potassium. If the slices are then incubated in aerobic conditions in the presence of glucose and glutamic acid the normal potassium content in the cells is restored. The same result is obtained in experiments on slices of kidney cortex. A similar picture was observed by Terner, Eggleston and Krebs (1950) and Davies and Krebs (1952) in experiments on brain slices and on retinae of guinea pigs. When the slices were incubated there was observed to be a rapid transfer of intracellular potassium into the surrounding medium. Upon subsequent addition to the medium of glucose and glutamic acid in the presence of oxygen restoration of the potassium in the tissues is discovered. In both cases sodium moves in the opposite direction to potassium. After the period of restoration of potassium in the cells, its concentration remains on a constant level for a lengthy period; but by means of radioactive potassium it has been established that rapid exchange takes place of intracellular potassium for potassium of the medium. The processes of the restoration of potassium in the cells require, as these authors observed, the presence of glutamic acid and the energy of oxidation of glucose. Tolulyl-n-sulphamide, which is an inhibitor of carbonic-anhydrase, according to their data, depresses the rate of accumulation of potassium in cells and causes a drop in its concentration in them.

A similar phenomenon was observed by Pulver and Verzár (1940) in experiments with yeast cells. The addition of sugar to a suspension of yeast cells leads to strong absorption by the cells of potassium, which the cells slowly begin to lose when the fermentation process has ended. Further, Hevesy and Nielsen (1941) discovered by means of radioactive potassium that besides the increased absorption of potassium by yeast cells during fermentation there also occurs rapid exchange of intracellular potassium for potassium of the medium. In contrast to the first two authors Hevesy and Nielsen came to the conclusion that the increased absorption and potassium exchange on fermentation are due not to increased permeability of the membranes, but to the synthesis of the polysaccharides with which the potassium binds. After the end of the fermentation process the polysaccharides decompose, the potassium is released and can move out of the cell and exchange rapidly for potassium of the medium.

In connection with these observations we should quote the material on the effect of insulin on the mineral exchange of cells. In our discussion of the permeability of muscle fibres for sugars we quoted data showing that insulin strongly raised the permeability of cells for a number of sugars. It was also noted that in the presence of glucose and insulin in the medium there is observed to be increased synthesis of glycogen. Besides this a number of authors have observed an increased potassium concentration in cells and a depressed sodium concentration. Such data have been obtained in experiments on isolated rat diaphragms (Flückiger and Verzár, 1954; Creese, D'Silva and Northover, 1958; Creese and Northover, 1961). Insulin facilitates the maintenance of a high potassium concentration in isolated rat and frog muscles when they are kept in saline solutions (Manery, Smillie and Toye, 1954; Zierler, 1957a, 1959, 1960; Randle and Smith, 1958a, b; Horowitz, 1959; Bhattacharya, 1959; Smillie and Manery, 1959; Ungar and Kadis, 1959; Kernan, 1961a). Insulin increases the rate of flux of sodium ions out of muscle fibres in comparison with the control muscles (Kernan, 1961b). In the presence of insulin in the medium there occurs not only a higher potassium concentration in the muscle fibres, but also a higher resting potential (Zierler, 1957a, 1959; Kernan, 1961a), and a lower concentration of free sulphhydryl groups of muscle proteins (Ungar and Kadis, 1959).

The influence of insulin on the maintenance by cells of a high potassium concentration can be explained as in the case of fermenting yeast cells by the fact that the potassium is bound by glycogen, whose synthesis is stimulated by insulin (Fenn, 1939; Conn and Wood, 1957). Further, cell proteins in the presence of insulin and glucose are less altered (denatured), as is witnessed by the considerable lowering of the free sulphhydryl groups in the cell proteins in the presence of insulin and glucose. This should facilitate the increased binding by the proteins of potassium in preference over sodium (Ungar and Kadis, 1959; Ungar, 1959, 1961a, b).

It should, however, be noted that the causes of enhanced potassium con-

centration in cells under the action of insulin still remain obscure, just as we are not clear about the causes of the enhanced permeability of cells for a number of sugars under the action of this hormone. It is known that the permeability of muscle fibres for sugars can be raised by many factors such as, for example, muscular work (Cori, Closs and Cori, 1933; Goldstein *et al.*, 1953; Helmreich and Cori, 1957; Sacks and Smith, 1958; Kipnis *et al.*, 1959; Sacks, 1960); the action of many cell poisons: anoxia (Randle and Smith, 1958b; Morgan *et al.*, 1959), sodium salicylate (Randle and Smith, 1958a; Sacks and Smith, 1958; Morgan *et al.* 1959), 2,4-dinitrophenol in aerobic conditions (Randle and Smith, 1958a, b; Morgan *et al.*, 1959), cyanide and sodium arsenate (Randle and Smith, 1958a, b), starvation (Kipnis *et al.*, 1959), elevated concentration of magnesium ions, while calcium ions depress the penetration of sugars (Bhattacharya, 1959), just as happens in the presence of phloretin (Morgan and Park, 1957; Crane, Field and Cori, 1957; Krane and Crane, 1959; Morgan *et al.*, 1959). On the other hand, besides potassium, insulin stimulates the absorption by cells of orthophosphate (Horowitz, 1959), oxygen (Manery, Smillie and Toye, 1954; Smillie and Manery, 1959) and certain amino acids (Kipnis and Noall, 1958; Manchester and Young, 1960), and the release from cells of aldolase (Zierler, 1957b, 1958). It is interesting that in certain conditions adrenaline too increases the accumulation by cells of potassium (Dresel and Wollheim, 1924; Born and Bülbring, 1956).

The loss by cells of potassium and the absorption of sodium, chloride and water is observed upon denervation of organs (Humoller *et al.*, 1950; Kobbinger *et al.*, 1955; Harris and Nicholls, 1956; Cort and Kleinzeller, 1956; Klass *et al.*, 1960), muscular dystrophy (Fenn and Goetsch, 1937; Williams *et al.*, 1957; Baker *et al.*, 1958; Young Ho Lee *et al.*, 1959), feeding of animals with small amounts of potassium (Heppel, 1939; Brokaw, 1953; Schwarz, Cohen and Wallace, 1953) or with food containing no proteins, as a result of which the protein content of the blood plasma falls (Hegsted *et al.*, 1953a, b). The placing of isolated organs in saline solutions containing proteins or in blood plasma prevents cells from absorbing sodium (Fenn, Cobb and Marsh, 1934; Opie and Rothbard, 1950; Carey and Conway, 1954; Creese, D'Silva and Northover, 1958; Creese and Northover, 1961). Loss of potassium by the muscle fibres and entry into them of sodium, chloride and water occurs when thiopentol is introduced into the blood, when the terminal blood vessels are squeezed or when a suspension of finely ground glass is introduced into them (Fuhrman, 1951a, b; Fuhrman and Crimson, 1951).

The above noted fact of the redistribution of potassium and sodium between cells and medium as a result of shifts in metabolism indicates the affinity of this phenomenon with that observed upon excitation and reversible damage. When there is insufficient oxygen and glucose in the medium, just as in the case of excitation and reversible damage, there is ob-

served, as we have seen, release of potassium from cells and uptake of sodium. The addition to the medium of glucose in aerobic conditions, just like the return of a cell from the excited state to the state of rest and the elimination of damage, is accompanied by the reverse movement of potassium into the cell and of sodium from the cell into the medium.

If there is every reason for explaining the redistribution of potassium between live matter and medium by a change in the properties of the organic compounds of the cell with which the potassium is bound, then the redistribution of sodium, which is induced by the same factors, is evidently caused by a change in the properties of the protoplasm as a solvent and by the increase in the affinity of proteins to sodium ions (Ling, 1957, 1960; Ungar, 1959, 1961 a, b). It is known that irritation of and damage to a cell leads to the evening out of the sodium concentrations in the cell and its surrounding medium. Because the sodium concentration in the medium and the concentration of its dissolved fraction in the cell are comparatively large (many times greater than the concentration of free potassium and other elements), a small rise in the solvent capacity of the protoplasm will be enough to cause the sodium to shift from the medium into the cell and its concentration in the latter to grow notably.

Of course, what we are speaking of here is a change in the solvent capacity of the protoplasm as a whole, and not only in respect of sodium. This is indicated by the following facts. Thus, Eichler and Schmeiser (1951) discovered that after several hours of passing a solution containing radioactive phosphate through an isolated working frog ventricle, when the amount of phosphate absorption reaches a constant level, stopping the availability of air leads not to reduced phosphate absorption, as was expected by these authors on the basis of the fact that the entry of phosphate into a cell is connected with its enzymatic inclusion into the phosphoric esters within the bounds of the cell membrane, but, on the contrary, to increased entry of phosphate into the heart muscle. There is every reason for supposing that the solubility of chloride and other ions in the protoplasm under anaerobiosis and the action of various metabolic inhibitors also increases, as it does upon excitation and damage.

The fact that anaerobiosis and the action of metabolic inhibitors produce deep changes in the properties of the cell colloids is shown not only by the fall in its "affinity" for potassium resulting in the release of the latter from the cell, but also by the increase in the adsorption (or chemical binding) of certain other substances, such as vital dyes.

As has already been said, Nasonov and Aleksandrov, and after them numerous other scientists, connect the increase in the colorability of the protoplasm and certain other phenomena that occur simultaneously, with alteration (reversible denaturation changes) in the cell proteins.

In their book Nasonov and Aleksandrov (1940) cite abundant experimental evidence from many sources which shows that "... increase in the color-

ability of live matter can occur not only as a result of the direct action of some agent on the protoplasm from outside, but also in consequence of breakdown of metabolism in the organism" (p. 87). Thus, Nasonov (1938) introduced a solution of neutral red into frog intestine which had been kept for 1·5–2 hr previously in an atmosphere of hydrogen and was then placed for another similar period in an atmosphere of hydrogen. The control frogs, into whose intestine the dye was also introduced, were kept in air. The cells of the intestinal epithelium of the experimental frogs showed strong diffuse colouring of the protoplasm, granule formation ceased and the nucleus was deformed and also strongly coloured; the type of colouration indicated a shift in pH towards the acid side. If the coloured frogs were transferred from hydrogen to air, the colour picture after a short time was the same as in the control: the nucleus became pale, granules of dye formed in the protoplasm, the colouring of the protoplasm also vanished. Nasonov (1930, 1932a) showed that during asphyxia (when the frogs were in the hydrogen atmosphere) there occurs a strong lowering of the intracellular redox potential.

A similar effect of asphyxia on the vital dye of cells of various tissues of the larvae *Chironomus* and *Daphnia* was observed by Aleksandrov (1932). Similar results were also obtained by Meshcherskaya (1935) in experiments with various insects.

Increased colorability by neutral red of the striated muscles of frogs was observed by Braun and Ivanov (1933) both in the whole organism when placed in an oxygen-free medium and in isolated muscles too. In the first case the experimental muscles bound the dye 2·5 times more than the control, in the second 2 times. Increased sorption of neutral red was observed by Gubler (1949) in the brain and muscle tissues of frogs upon oxygen starvation. The data of Nasonov were confirmed by Gersch (1936) in the colouring with neutral red of infusoria in conditions of asphyxia. The colouring of plant cell nuclei by neutral red under asphyxia was observed by Becker and Beckerowa (1934).

Makarov (1934b, 1935b) caused paranecrotic type of colouring of the cytoplasm and nucleus by subjecting the cells of frog intestinal epithelium and infusoria to carbon monoxide gas. Meshcherskaya-Sheinberg (1939) observed strong, but reversible, colouring and luminescence of the nucleus of fish and frog erythrocytes when subjected to respiratory poisons (cyanides and azides) and also upon the exclusion of air.

Colouring of live cells of the paranecrotic type can occur in neutral conditions if there is insufficient oxygen. In this case the colouring of the nucleus and protoplasm, like the other signs of paranecrosis, is determined by the change in the metabolic system. As was shown by Nasonov (1932b), a large amount of anaerobic infusoria, which obtain their energy from glycolysis and not respiration, in 1–2 min after immersion in a 0·025 per cent solution of neutral red show very strong colouring of the macronucleus, whereas the macronucleus of aerobic infusoria is not coloured. The shade

of colouring of these last indicates a more basic reaction than the reaction in anaerobic infusoria.

Of great interest are the observations of Aleksandrov (1932) on the mosquito larvae *Chironomus plumosus* which live in mud in conditions of pronounced oxygen deficit. They sometimes rise to the surface layers of the water, into a medium with adequate oxygen, and in these conditions become aerobic organisms. The author found that the colouring of the tissues of this organism by neutral red in mud is of the paranecrotic type, just the same as is observed in larvae taken from the surface of the water when placed in an atmosphere of hydrogen (in asphyxia).

This change in type of colouring is very well expressed in such optional anaerobes as yeast cells. Meisel (1938b) showed that if yeast cells are placed in conditions when respiration is replaced by fermentation, then the cells increase in size, the protoplasm and nucleus begin to luminesce on a dark ground and are strongly coloured by neutral red, while in oxygen respiration this does not occur. As Meisel showed, the protoplasma of fermenting cells binds neutral red and fluorochrome 25–35 per cent more than the same yeast cells when respiring. The increased binding of neutral red by fermenting cells is connected by this author with the increase in the cells of free protoplasma nucleic acid, of which there is 30 per cent more than in respiration cells.

In their book Nasonov and Aleksandrov (1940) cite a vast amount of published work which shows that a change in the metabolic system or a change of its normal course under the action of many factors changes the sorptional properties of live matter with respect to dyes.

Nasonov (1930, 1932a) supposed that the change in live matter upon asphyxia (paranecrosis) is a consequence of the action on the cell colloids of incompletely oxidised products of metabolism.

Thus, the experimental data quoted show that to maintain certain physico-chemical properties of the cell colloids which can ensure some particular form of physiological activity of the protoplasm, including the permeability of cells, on a determinate level, it is essential that there should be an uninterrupted flow of energy of metabolism. In this connection it is essential to remember that Sabinin (1940), analysing a large amount of data indicating the role of respiration in the process of the mineral nutrition of plants, came to the important conclusion that "respiration is the process which causes the activation of the molecules of the components of the protoplasm which form compounds with the substances that penetrate the cell" (p. 72).

This conclusion is in agreement with all the facts quoted above and also with the widespread view of the protoplasm as a special physico-chemical structure which exists only thanks to the constant expenditure of metabolic energy and which is thus capable of displaying biological, physiological and biochemical activity. This structure is supported "actively", in Kursanov's

expression (Kursanov, 1940), "compulsorily" in the sense that this involves the consumption of a definite amount of energy of intracellular metabolism. According to Lepeschkin (1930, 1936, 1937), metabolic energy is essential for effecting and maintaining the binding of proteins with lipids (to ensure the "vitaids" structure of the protoplasm). According to the ideas of Nasonov and his colleagues (Nasonov and Aleksandrov, 1940; Aleksandrov, 1951; Nasonov, 1951 etc.) part of the energy of metabolism is expended on the preservation of the protoplasmic proteins in their native state; under the action of various irritants these are altered (reversibly denatured), and to return them to their initial state the expenditure of metabolic energy is essential. It is intelligible that the point of view of Nasonov and his colleagues does not contradict the hypothesis of the "vitaids" structure of live matter put forward by Lepeschkin. Equally, it does not contradict the hypothesis of the coacervate structure of the protoplasm, first put forwards by Bungenberg de Jong and to-day adopted by many scientists. On the contrary, it points to the basic principle underlying the existence of the characteristic structure of the protoplasm (lipoprotein complexes—"vitaids"—complex coacervate—and possibly some other structure).

A similar position in this problem is to-day taken by a number of workers (Ungar, 1957, 1959, 1961a, b; Segal, 1958; Epshteyn, 1959; Simon, 1959; Kometiani, 1956, 1961a, b).

The connection between metabolism and cell permeability also shows in the fact that various factors in the external medium which somehow or other affect the permeability of the cell at the same time affect the work of the cell enzymes too, changing the direction of their activity, as has been shown by Oparin and his colleagues in the development of their theory of the action of enzymes in the cell.

According to this theory, enzymes can exist in the protoplasm in two forms: in the free (dissolved) and in the bound (adsorbed on to the structural elements of the protoplasm) states. In the dissolved state the enzymes are hydrolytically active and effect the splitting of substances, while in the bound state they lose this capacity and acquire a synthetic action (Kursanov, 1940; Oparin, 1948; Sisakyan, 1951 etc.).

The state of the enzymes in a cell is directly related to the sorptional capacity of the protoplasmic structures, and "... whether the change in the adsorptional capacity of the protoplasm occurs under the influence of external agencies or in the course of the normal life cycle, this change is always completely determinately tied up with the change in the activity of the protoplasmic enzymes proper. It is just the increase in adsorption that always leads to a parallel weakening in the hydrolytic activity of various enzymes, and simultaneously with this there occurs increased synthesis of polysaccharides, proteins and so on" (Oparin, 1948, p. 15).

The causes producing these changes in the sorptional properties of the protoplasm and the direction of the enzyme process can vary widely. This

occurs when the same agencies act on the cell as those which cause parabiosis and paranecrosis (Kursanov and Kryukova, 1937b; Kursanov, Kryukova and Morozov, 1938; Sisakyan and Kobyakova, 1938, 1949; Oparin, 1948; Sisakyan, Zolkover and Biryuzova, 1948; Sisakyan, 1951 etc.).

From a comparison of the data obtained by Nasonov and other workers on the change in the sorptional properties of live matter under asphyxia, respiratory poisons and other factors with those obtained by Oparin and his colleagues, we should come to the conclusion that those changes in the cell which were labelled paranecrotic by Nasonov and Aleksandrov play the decisive role in the change of metabolism, in the change of the direction of enzymatic reactions. These same changes in the physicochemical properties of the cell colloids are probably the trigger for the biochemical reactions which ensure some or other physiological reaction which takes place in response to an external irritation (see also Frank, 1960).

Thus, the problem of the unity of structure and function in the quoted work of cytophysiologists and biochemists finds its experimental solution. Paranecrotic changes in live matter serve as "... the trigger mechanism for a chain of complex biochemical reactions which result in the bringing about of some cell activity" (Nasonov and Suzdal'skaya, 1948, p. 409).

For muscles this activity expresses itself in mechanical work, for nerve conductors in the conduction of nerve impulses, for a glandular cell in the discharge of secretion and so on.

Simultaneously there is a change in the permeability of the cell, in some cases upwards and in others downwards.

#### 4. *Conclusions*

1. There is a direct connection and mutual dependence between metabolism and cell permeability.

2. For those substances which take direct part in enzymatic reactions as a result of which they are converted into other substances and come to form part of the protoplasm or are excreted, the permeability of a cell is greater the greater the rate of the enzymatic reactions.

3. This conclusion is valid upon the condition that the concentration of the substrate of the enzymatic reactions penetrating the cell is sufficiently high (physiological optimum or higher). At low concentrations of these substances in the medium, the permeability of the cell for them increases thanks to the adsorptional or chemical binding of them by the cell colloids. This evidently ensures adequate inflow of the substances into the cell and maintains the optimum rate of the enzymatic reactions.

4. Thanks to metabolism, the sorptional activity of the protoplasm is maintained on a definite level. Upon breakdown of metabolism (insufficiency of oxygen, action of metabolic inhibitors), as upon excitation and damage, this level changes: the solubility of substances in the protoplasm increases

and the binding by the cell colloids of some substances is depressed (potassium) and of others is enhanced (vital dyes, possibly, sodium ions). As a result of this there occurs a redistribution of substances between cell and medium, and the permeability of the cell for some substances increases (sodium, chloride, vital dyes, etc.), and for others decreases (potassium, phosphate, etc.).

5. The process described in the preceding paragraph is in certain conditions reversible. The restorative process can be achieved at the expense of a definite amount of metabolic energy.

6. The permeability of a cell for any substance is always a physiological phenomenon, because the rate of penetration of substances into a cell and their distribution between cell and medium are determined by the rate of the enzymatic process directly or thanks to the fact that metabolism maintains the sorptional activity of live matter on a definite level (the solubility of substances in the protoplasm and the adsorptional and chemical binding of them by the cell colloids). The division of the permeability of cells into "physiological" (when the absorption of substances by a cell is achieved actively, with the expenditure of energy) and "physical" (when the penetration of substances into a cell is not accompanied by the expenditure of metabolic energy) finds no experimental foundation.

## CHAPTER XI

# The Bioelectric Properties of Cells

### 1. *On the Nature of Bioelectric Potentials\**

The theories generally held to-day about the causes of bioelectric potentials are based on the asymmetry of the distribution of ions between cell and medium. The ionic nature of bioelectric currents is indicated by the vast accumulation of factual data which has served as the basis of the membrane and phase theories of bioelectric phenomena. The first scientist to give an orderly physico-chemical foundation to the origin of bioelectric currents was Chagovets (1896, 1903), who used the theory of electrolytic dissociation of Arrhenius (1887). Here we shall discuss the membrane and phase theories only with reference to the data on cell permeability quoted in the preceding chapters. A detailed exposition of the modern membrane theory of bioelectric potential differences is given by Hodgkin (1951, 1958), while the phase theory of these phenomena is set out in a book by Nasonov (1959 a). We shall not go into the details of these theories here.

#### A. *The membrane theory*

*Resting potential.* The physical foundations of the possibility of the formation of membrane potentials was given by Ostwald (1890). A detailed study of these potentials was made by Michaelis *et al.* (1925-7) in experiments with artificial membranes. The basic propositions of the classical membrane theory were formulated by Bernstein (1902, 1912) and developed in a series of papers by Höber (1905, 1908, 1926, 1945) and other writers.

Bernstein's hypothesis was based on the following premises: (1) the surface layer of the protoplasm is a special structure—a membrane permeable only for potassium ions (and other cations of smaller radius); (2) all the rest of the protoplasm is an aqueous solution in which mineral substances, including potassium, are present in the free and ionised state; (3) the potassium concentration in the protoplasm is considerably greater than in the external solution.

\* In writing this section of the chapter I have received much help from my colleagues in the Institute of Biological Physics of the Academy of Sciences of the U.S.S.R. (Moscow), Ye. M. Liberman and L. M. Chailakhyan, to whom I am profoundly grateful.

Consequently, there arises a concentration gradient for potassium ions across the cell membrane, and it is this that determines the magnitude of the membrane potential differences (PD) according to the equation:

$$\Delta V = - \frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_o}, \quad (1)$$

where  $\Delta V$  is the potential difference between the outside and inside surfaces of the membrane,  $R$  the gas constant,  $T$  the absolute temperature,  $F$  Faraday's number,  $[K^+]_i$  and  $[K^+]_o$  the potassium concentration inside and outside the cell respectively. Equation (1) is the limiting case of the Nernst diffusion equation, when the mobility of the anions is equal to zero.

Later, however, in the 1930's it was demonstrated by a number of authors (see Ch. IX) that excitable tissues are completely permeable for chloride ions. In this connection Boyle and Conway (1941) put forward the suggestion that the membrane potential is a Donnan potential and is active not only for potassium ions but for chloride also, so that:

$$\Delta V = - \frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_o} = \frac{RT}{F} \ln \frac{[Cl^-]_o}{[Cl^-]_i}, \quad (2)$$

the notation being the same as in equation (1). The Boyle and Conway hypothesis preserves Bernstein's basic idea of the role of the ionic gradient through the membrane in the formation of the resting current.

But, as we have already seen, the cell is permeable not only for potassium and chloride ions, but for sodium too (see Ch. IX). This fact, discovered with the aid of radioactive sodium ions at the end of the '30's, contradicted not only the assumptions of Bernstein and Boyle-Conway, but also the entire membrane concept as a whole as an attempt to explain the asymmetry in the distribution of sodium ions between cell and medium by the impermeability of the cell membrane for these ions. In this connection the idea arose (Dean, 1941a) of the active expulsion from the cell of sodium, which passes into it passively along the concentration gradient (the sodium pump, see Ch. X). From that time there arose in physiology concepts of two kinds of ion distribution between cell and medium: a passive, caused by diffusional and electrical forces, and an active, founded on special membrane mechanisms operating at the expense of the metabolic energy in the cell.

If, as Hodgkin (1951) indicated, the work of the sodium pump in expelling sodium balances the entry of sodium ions into the cell along the gradient, then the passive distribution of potassium and chloride ions should be exactly the same as it would be taken to be from the assumption that sodium ions are absolutely incapable of penetrating the membrane and would remain thus in virtue of the Boyle-Conway hypothesis concerning ion distribution.

Thanks to the development of methods of investigation on single muscle and nerve fibres, and also thanks to the development of the microelectrode technique of measuring resting and action potentials (Ling and Gerard, 1949), true values were evidently obtained for the resting potentials, which varied within the limits 50–100 mV. This made it possible to find out whether the resting potential (or transmembrane potential) was a potassium potential. It was discovered that the value of the resting potential as calculated from the Nernst formula (equation 1) was higher than that found experimentally, being 85–105 mV. Such divergences are explained by the fact that the cell membrane is permeable for sodium and that isolated tissues in artificial media lose potassium and acquire sodium (see Ch. X), that is, the cells are not in a state of equilibrium ion balance. In such conditions the membrane potential is a function of the relative permeability and the concentration gradients of all the ions which can cross the membrane, i.e., in these conditions it cannot be the potassium electrode potential.

In view of this, and working from the equation of Goldman (1943), according to which the field strength inside the membrane due to a suitable distribution of stationary charges is constant, Hodgkin and Katz (1949a) derived the following equation for the membrane potential difference:

$$\Delta V = - \frac{RT}{F} \ln \frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}, \quad (3)$$

where  $P_K$ ,  $P_{Na}$  and  $P_{Cl}$  are the permeability constants of the membrane for ions of potassium, sodium and chloride, the letters in square brackets indicating the ion concentrations inside (i) and outside (o) the fibre.

The resting current for a single squid nerve fibre calculated from this formula coincides almost exactly with the observed, if it is assumed that  $P_K : P_{Na} : P_{Cl} = 1 : 0.04 : 0.45$ . The observed PD is 61–62 mV, and the calculated value is 59.5 mV.

On the basis that the physical significance of the difference in the permeability of the membrane for different ions can be reduced to the difference in the energy barriers for the passage of these ions into the membrane, Liberman (1961) made a detailed theoretical analysis of equation (3) and showed the limits of its applicability.

*Action Potential.* One of the first hypotheses concerning the causes of action potentials to obtain widespread recognition was that of Bernstein (1902, 1912). His idea was that the action potential (AP) is inseparably linked with the resting potential. Bernstein supposed that at the moment of excitation the cell membrane becomes permeable for all ions. In view of this the membrane potential in the excited section of the nerve or muscle fibre falls to zero and the potential energy of the ionic gradient becomes a rapidly moving ionic current.

Certain essential features of Bernstein's hypothesis were later confirmed experimentally. Cole and Curtis (1938, 1939) demonstrated that the permeability of muscles and nerves is enhanced upon excitation, and a number of authors (see preceding chapter) established that a redistribution of ions between cells and medium takes place upon excitation. However, new facts were later obtained which did not fit into the framework of the classical theory of bioelectric currents, in which connection this theory was subjected to searching criticism by a number of physiologists (Arshavskii, 1939; Lebedinskii, 1939; Nasonov, 1939, 1949a; Nasonov and Aleksandrov, 1944, 1950; Makarov, 1949, etc.). Thus, according to Bernstein, the action potential cannot in any event exceed the resting potential. This notwithstanding, it has recently been shown that the action potential is as a rule higher than the resting potential by 1·5–2, and in some cases even 3, times. Hodgkin and Huxley (1939) and Hodgkin and Katz (1949a) discovered in the case of a single squid nerve fibre that the maximum resting potential is about 50 mV, and the action potential 90 mV. Curtis and Cole (1942) give even larger values: on the average 61 and 108 mV respectively, though in some cases the action potential reached as much as 168 mV. Giant cuttlefish nerve fibres give an average resting potential of 62 mV and an action potential of 120 mV, some fibres actually giving 164 mV (Weidmann, 1951). Such excess of the action potential over the resting potential has been described by many writers in the case of the nerve conductors of other animals, and also in muscles, as can be seen from Table 101.

This same feature was observed also by Graham and Gerard (1946) and Stephenson (1953b) in frog skeletal muscle fibres, by Trautwein, Zink and Kayser (1953) and Woodbury and McIntyre (1954) in muscle fibres of humans and mammals in experiments conducted *in vivo* with the introduction of a microelectrode into the muscle fibre, by Woodbury *et al.* (1951) in muscle fibres of frog heart, by Fing *et al.* (1951) in embryonic muscle fibres of chicken, by Stephenson (1953a) in muscle fibres of crab feet and by Woodbury (1952) in nerve fibres of frog sciatic nerve. In later work done on a wide variety of preparations by many workers it was also established that the AP is greater than the RP; the values obtained are close to those quoted in Table 101 (Brock *et al.*, 1952; Ling, 1952; Trautwein, Gottstein and Dudel, 1954; Draper and Tu, 1955; Frankenhaeuser, 1957; Keynes and Swan, 1959b; Moore and Cole, 1960; Kernan, 1960; Kostyuk, 1957; Sorokina, 1961a, b, etc.).

Many hypotheses were advanced to explain the fact of the excess of the action potential over the resting potential with a view to modernising the classical membrane theory of bioelectric potentials. A complete cross-section is to be found in the reviews of Grundfest (1947) and Hodgkin (1951). The possibility was assumed that on the depolarisation of the membrane during excitation the ions are somehow desolvated, which should lead to a considerable increase in the rate of diffusion of sodium and to a considerable

retardation of potassium. The possibility was also assumed of the penetration of sodium in the undissociated form in combination with molecules of the membrane, and so on.

According to the hypothesis of Danielli (1941), there occurs during excitation disorientation of the lipid molecules in the inner layer of the membrane, which consists of two layers of lipid molecules with their ionised ends

TABLE 101. THE VALUE OF RESTING AND ACTION POTENTIALS (RP and AP in mV).

Animal	Tissue	RP	AP	Author
<i>Loligo forbesi</i>	non-medullated nerve	50	90	Hodgkin and Huxley, 1939
<i>Loligo forbesi</i>	non-medullated nerve	48	88	Hodgkin and Katz, 1949a
<i>Loligo pealii</i>	non-medullated nerve	51	104	Curtis and Cole, 1942
<i>Sepia officinalis</i>	non-medullated nerve	62	120	Weidmann, 1951
<i>Sepia officinalis</i>	non-medullated nerve	—	124	Weidmann, 1951
<i>Homarus vulgaris</i>	non-medullated nerve	—	110	Hodgkin and Rushton, 1946
<i>Homarus vulgaris</i>	non-medullated nerve	62	106	Hodgkin and Huxley, 1945
<i>Carcinus maenas</i>	non-medullated nerve	—	116	Hodgkin, 1947
<i>Carcinus maenas</i>	non-medullated nerve	71–96	116–153	Hodgkin and Huxley, 1945
<i>Rana esculenta</i>	sciatic nerve	71	116	Huxley and Stämpfli, 1951
<i>Rana temporaria</i>	skeletal muscle	88	119	Nastuk and Hodgkin, 1950
<i>Rana pipiens</i>	heart muscle	50–90	65–115	Woodbury, Woodbury and Hecht, 1950
<i>Canis familiaris</i>	heart, Purkinje's fibres	90	121	Draper and Weidmann, 1951
<i>Capra hircus</i>	heart, Purkinje's fibres	94	135	Draper and Weidmann, 1951

pointing in opposite directions (towards the "aqueous" phases, i.e. towards the protoplasm and towards the surrounding medium). In the state of rest this membrane is electroneutral, and, when its inner layer is disorientated, the external surface will be strongly negatively charged with respect to the inner layer, which will now be positively charged. This hypothesis was defended by Rubinstein (1949b) as being the most probable solution of the problem of bioelectric potential in the framework of the membrane theory; it has not, however, found much support among physiologists.

A similar kind of hypothesis to explain the predominance of the action potential AP over the resting potential RP was advanced by Hertz (1947). Holding as he did to the views of Lundegårdh and Krogh about the structure of the plasma membrane, he supposed that in the excited section of the nerve or muscle fibre the membrane micelles are revolved through 180°, as a result of which the membrane at this point acquires an electrical charge of the opposite sign.

Other hypotheses were also advanced which have also been abandoned and are now of historical interest only (Curtis and Cole, 1942; Hodgkin and Huxley, 1945, and others: see the review of Liberman and Chailakhyan, 1959).

To explain the predominance of the action potential over the resting potential, Hodgkin and Katz (1949a) put forward the "sodium hypothesis" which has obtained wide circulation and recognition. The basic idea of this hypothesis is that upon excitation what happens is not a simple general enhancement of the permeability of the cell membrane, as Bernstein supposed, but a specific enhancement of the permeability for sodium, which should be observed only during the rising part of the spike, after which the permeability of the membrane for potassium should rise.

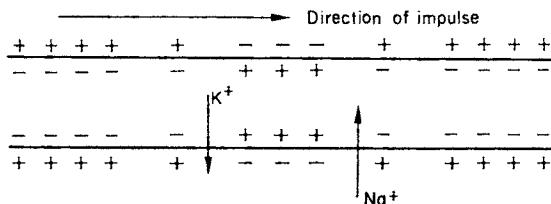


FIG. 122. Scheme of the movement of ions of potassium and sodium across the fibre membrane and its change of charge upon excitation (from Hodgkin, 1951).

According to this hypothesis, the cell membrane in the state of rest is most permeable for potassium ions and behaves in the first approximation as a potassium electrode, and the magnitude of the membrane potential is determined by equation (1). If, as Hodgkin and Katz supposed, the permeability of the membrane for sodium ions suddenly rises 500 times, then the membrane potential according to equation (3) will now be determined by the sodium ion gradient: the membrane in the first phase of excitation will behave as a sodium electrode and the membrane potential now takes the opposite sign (see Fig. 122), its value being given by the equation:

$$\Delta V = - \frac{RT}{F} \ln \frac{[Na^+]_i}{[Na^+]_o}, \quad (4)$$

where  $[Na^+]_i$  and  $[Na^+]_o$  are the sodium concentrations inside and outside respectively.

At the time when the sodium hypothesis appeared there was an accumulation in the literature of facts that were compatible with this hypothesis. Thus, as early as Overton (1902b) it was discovered that muscles lose their excitability in a medium where the sodium ion concentration is reduced to about a tenth of normal. Overton at that time suggested that there should occur upon excitation an exchange between cell potassium and external sodium. Later Overton's observations were confirmed on different subjects by a number of workers (Kato, 1936; Erlanger and Blair, 1938; Webb and Young, 1940; Lorento do No, 1947; Feng and Liu, 1949; Draper and Weidmann, 1951; Huxley and Stämpfli, 1951). It has also been shown that lowering the concentration of sodium ions has practically no effect on the magnitude of the resting potential, but lowers the overshoot of the action potential proportionally to the logarithm of the external sodium concentration (Hodgkin and Katz, 1949a; Feng and Liu, 1949; Nastuk and Hodgkin, 1949, 1950; Draper and Weidmann, 1951; Desmedt, 1953). In experiments with giant squid fibres it has been shown that raising the sodium ion concentration inside the fibre by micro-injection lowers the overshoot of the action potential over the resting potential (Hodgkin and Keynes, 1956). It has also been noted by a number of authors that the rate of the growth phase of the AP is in the first approximation proportional to the sodium ion concentration in the external solution. In the preceding chapter we have quoted data from many authors showing that upon excitation there takes place an exchange of cell potassium for sodium of the medium equal approximately to  $4 \text{ pm/cm}^2/\text{impulse}$ . This also provides support for the sodium hypothesis, if it be assumed that the movement of sodium ions inwards and of potassium ions outwards takes place at different times, as otherwise there would be no change observed in the membrane potential (Hodgkin and Katz, 1949a; Hodgkin, 1951, 1958; Eccles, 1953; Liberman and Chailakhyan, 1959).

Until very recently nobody had succeeded in obtaining direct verification whether in fact upon excitation a sodium ion flux into the cell is initially observed, which is subsequently replaced by a potassium flux out of the cell and restores the polarisation of its surface. In the opinion of the majority of physiologists, the development by Cole (1949) and Marmont (1949) of a technique of "fixation" of the membrane potential at a definite level ("voltage-clamp method") has made it possible to study these fluxes indirectly. Using this method in experiments with squid giant fibres Hodgkin, Huxley and Katz (1952; also Hodgkin and Huxley, 1952a, b, c, d) obtained a number of facts which were interpreted as a proof of the correctness of the sodium hypothesis.

It was shown (Hodgkin, Huxley and Katz, 1952) that if the membrane potential is lowered below the threshold value, then there is at first observed a short-lived (about 2 msec) ion current into the fibre. This current quickly

drops and is replaced by a current outwards which continues for several dozen msecconds. The maximum values of the inward current and outward current differ for different values of the membrane potential.

According to Hodgkin and Huxley (1952a), the initial inward ion current can be treated as a sodium ion flux into the fibre because it disappears only when the voltage on the membrane corresponds to formula (4). This relation is well enough confirmed upon the change of the external sodium ion concentration. The second phase of the ion current, which is directed outwards and grows more slowly, could be related to the potassium ion flux from the fibre into the medium. This might find support from the results of experiments in which the current upon long depolarisation was compared with the release of labelled potassium from the fibre (Hodgkin and Huxley, 1953). To explain the dependence of the "sodium current" on the membrane potential and its reduction with time, and also the absence of an initial current directed into the fibre, in a medium where the sodium was replaced by choline (Hodgkin and Huxley, 1952a), Hodgkin and Huxley (1952c, d) suggested the additional hypothesis of the transfer of sodium ions across the membrane by special "carrier" molecules which are "inactivated" with time as the AP is generated. Evidently these "carriers" are of a different kind from those whose presence in the membrane is postulated by the sodium pump hypothesis.

Thus, a series of these experiments apparently made it possible to determine the sodium and potassium currents separately, which would be a strong argument in favour of the sodium hypothesis. However, the changes in the ion fluxes observed upon fixation of the membrane potential can be explained also by the appearance in the "membrane" of two oppositely directed electromotive forces of a different kind, for example, chemical reactions on the surface of the fibre not connected with the processes of change of permeability for sodium and potassium. In this case, the division of the ion current into sodium and potassium currents will be incompetent, and the subsequent calculations of the sodium and potassium currents will also be illegitimate (see Liberman and Chailakhyan, 1959, 1961). This doubt, as we shall see below, was completely justified when direct observations could be made of the ion fluxes during the excitation wave.

The sodium hypothesis with the additional hypothesis of "inactivation of sodium carriers" also made it possible to explain such properties of excited tissues as the threshold, "all or nothing" reaction, accommodation and refractivity (see the work cited in this section of Hodgkin, Huxley and Katz and also of Liberman and Chailakhyan).

However, in recent years there has been a great accumulation of facts which stand in sharp contradiction to the sodium hypothesis. We shall consider some of these facts. It was discovered that the magnitude of the overshoot of the AP over the RP is not directly related to the sodium ion concentration in toad skeletal muscle fibres (Shaw and Simon, 1955a; Shaw, Simon and Johnstone, 1956; Shaw, Simon, Johnstone and Holman, 1956, etc.).

The replacement of the sodium ions in the medium not only by lithium ions, but also by such ions as tetraethylammonium and certain other quaternary ammonium ions, does not prevent the generation of the AP by frog nerve fibres (Lorento do No, 1947, 1948, 1949). Later the generation of the AP in the absence of sodium ions in the external solution was demonstrated in a series of works on a variety of subjects—on frog myelinated nerves (Larramendi, Lorento do No and Vidal, 1956; Lorento do No, Vidal and Larramendi, 1957; Lüttgau, 1958), mammalian nerve fibres (Sugayra and Laget, 1958; Greengard and Straub, 1958, 1959), smooth and striated muscles (Burnstock and Straub, 1958; Daniel and Singh, 1958; Woodbury and Goto, 1959; Koketsu and Nishi, 1960), in the study of nerve-muscle transmission (Fatt and Katz, 1953; Koketsu and Nishi, 1959) and in frog spinal ganglia (Koketsu, Cerf and Nishi, 1958a, b, 1959a, b).

Fatt and Katz (1953) found that crab muscle fibres which have been pre-treated with tetrabutylammonia give extended action potentials (EAP) in the absence from the medium of all univalent cations. In the same subject EAP of high amplitude (120–160 mV) are obtained in pure isotonic solutions of barium or strontium chloride (Liberman, Tsofina and Vaintsavaig, 1961; Liberman and Tsofina, 1961).

After treatment with potassium acetate solution, single giant squid fibres yield EAP in a medium free of sodium ions (Segal, 1958). In certain conditions EAP are obtained in single nodes of Ranvier in the complete absence from the medium of sodium and potassium ions (Mueller, 1958a, b, c).

In the muscles of crustacea, after prolonged incubation in tetraethylammonia solutions, EAP with an amplitude of 90–120 mV are observed. The spike amplitude was dependent on the calcium ion concentration in the medium and not on the sodium and magnesium ion concentrations. Similar prolonged spikes were also obtained without treatment of the muscles with tetraethylammonia in solutions containing high concentrations of strontium or barium chloride (Fatt and Ginsborg, 1958).

In the work quoted above, and also in a number of other works (Lyudkovskaya and Kayushin, 1959; Falk and Landa, 1960a, b etc.) it has been shown that it is possible to obtain EAP of various forms, in some cases lasting several minutes. Tasaki and his colleagues investigated the duration of the action potential in single nodes of Ranvier (Tasaki, 1955, 1956, 1957; Tasaki and Franke, 1955; Tasaki and Freygang, 1955) and in the giant fibres of cephalopod molluscs (Tasaki and Hagiwara, 1957); they discovered that the membrane resistance during EAP is significantly different from the resistance of the resting membrane only during the rising part of the spike, and that the resistance on the plateau of the EAP is little (only 10 per cent) different from that of the resting membrane, while it should drop to a minimum of a half. This fact is in direct contradiction to the sodium hypothesis.

From their study of the EAP, Tasaki and his colleagues brought out in opposition to the sodium hypothesis the hypothesis that there are in the cell

membrane two stable potential levels, each of which has corresponding to it a definite force and resistance (Tasaki and Hagiwara, 1957; Tasaki and Bak, 1958a, b; Tasaki and Spyropoulos, 1958). This hypothesis has not obtained widespread acceptance among physiologists.

Finally, in a series of works (Mueller, 1958a; Stämpfli, 1958, 1959; Segal, 1958; Tasaki, 1959) on myelinated and non-myelinated fibres hyperpolarising responses were discovered in solutions with a high potassium content. Spyropoulos and Tasaki (1960) consider that this whole group of facts from the work cited above stands in direct contradiction to the sodium hypothesis.

Very weighty arguments have recently been brought to bear against the sodium hypothesis by Spyropoulos, Tasaki and Hayward (1961). They developed a method which enabled them to make fractional determinations of the ion fluxes out of excited units during the action potential. In experiments with cells of *Nitella* they demonstrated that the outward flux of radioactive potassium, rubidium and caesium ions that have previously been introduced into the cells, rises in parallel with the rising part of the action potential and falls during the fall off phase. In experiments with single squid axons pretreated with tetraethylammonia, EAP lasting several seconds were obtained. In these conditions it was found that the potassium and sodium efflux grows strongly in the rising and falling phases of the action potential, but during the plateau the fluxes of these ions are the same as in the resting fibre. These facts are not amenable to explanation within the framework of the sodium hypothesis.

A weighty argument in favour of the membrane theory of the origin of bioelectric currents is the fact that the magnitude of the RP is in the first approximation determined as the potential of a potassium electrode—according to Nernst's formula (Equation 1). The magnitude of this potential is linearly dependent on the logarithm of the concentration of potassium ions in the medium (Curtis and Cole, 1942; Ling and Gerard, 1949; Nastuk and Hodgkin, 1950; Hodgkin, 1951, 1958; Conway, 1957; Jenerick, 1953; Desmedt, 1953; Harris and Martins-Ferreira, 1955; Adrian, 1956; Baker, Hodgkin and Shaw, 1961; Chailakhyan, 1959 and others). However, this dependence occurs in a range of potassium concentrations in the medium which is considerably higher than the physiological; this dependence is not observed in the concentration range from 0 to 10–15 mm/l and, consequently, is not a potassium potential (Lorento do No, 1947; Kostyuk, Sorokina and Shapovalov, 1959; Sorokina, 1959a, b; 1961b; Liberman, Tsofina and Vaints-vraig, 1961).

Further, it is known that the magnitude of the RP of nerve and muscle fibres determined experimentally is always lower than that calculated from formula (1) as the magnitude of the RP of a balanced potassium potential (see Ling, 1952; Grundfest, 1955; Hodgkin, 1958; Kernan, 1960). Artificial displacement of the potassium concentration in cells to either side leads to a change in the magnitude of the resting potential, but this change does not

correspond either to Nernst's formula (equation 1) or to Goldman's equation (equation 3). Thus, Tobias (1948a, b, c, 1950) showed that frog muscles which have been soaked in distilled water and lost their potassium almost completely, preserve a resting potential which is sometimes equal to 39 per cent of normal. According to Stephenson (1953a), isolated crab muscles incubated in sea water quickly lose a large amount of potassium and take up sodium; the resting potential remains unchanged the while. Elsewhere (Stephenson, 1953b, 1957) this worker showed that frog sartorius muscles, which have been kept for 24 hr at 2–3° in a potassium-free saline solution lose potassium and take up sodium. If the muscle is then placed in a saline solution containing potassium ions at room temperature, the muscle releases sodium ions and takes up potassium ions. This proved that when the muscle loses potassium, the resting potential remains unchanged, but while it takes up potassium, the resting potential not only does not grow, but actually falls. Lack of correspondence of the change in the resting potential to Nernst's formula was shown upon the treatment of rat skeletal muscles with insulin (Zierler, 1959).

It has been demonstrated by a number of workers that the introduction by micropipette into nerve or muscle fibre of univalent cations (potassium, sodium, rubidium and lithium) and of a number of anions does not lead to a change in the resting potential, whereas the introduction into fibres of very small amounts of bivalent cations causes a sharp lowering of the resting potential and a conduction block (Grundfest *et al.*, 1954; Falk and Gerard, 1954; Hodgkin and Keynes, 1956; Kostyuk, Sorokina and Shapovalov, 1959; see references in Grundfest, 1955). Finally, Koketsu and Kimura (1960) showed that frog sartorius muscles placed in a hypotonic solution of pure sucrose (112 mM) for various periods at various temperatures, lose potassium to a level of 20 per cent of normal but maintain normal resting and action potentials.

Hodgkin and Horowicz (1960a) in experiments with isolated frog muscle fibres found that the changes in the resting potential caused by sudden drops in the potassium concentration in the medium were less than the changes caused by raising the concentration of this ion or changing the chloride ion concentration in either direction. They note the lack of correspondence of their data with Nernst's equation and, to explain this phenomenon, suggest an additional hypothesis, "short-term holding" of potassium ions in a special region of the muscle fibre.

Thus the facts quoted, which are in contradiction to the membrane theory of bioelectric currents, compel us to seek other means of explaining this phenomenon.

### B. The phase theory

The phase theory of bioelectric potentials was formulated by Nasonov and Aleksandrov (1943, 1944; see also Nasonov and Aleksandrov, 1950; Nasonov, 1949a, 1959a). It is based on the idea that the disperse medium of the protoplasm behaves as a phase with respect to the surrounding solution of substances and that the greater part of the electrolytes in the protoplasm (normally and in the state of rest) is in the bound and not the dissociated state.

According to this theory, electrolytes are released and go into solution in the damaged or excited zone of the protoplasm; on making contact with the intact (resting) part of the protoplasm, these form a phase boundary potential (Fig. 123). Hence it follows that damage and excitation potentials are the

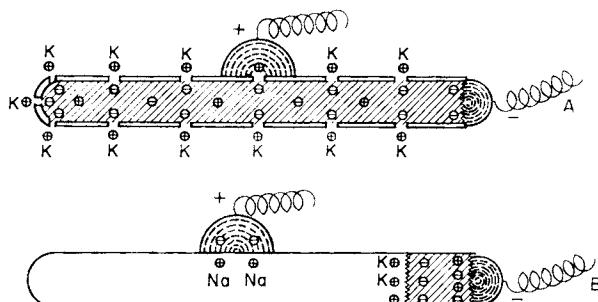


FIG. 123. Scheme showing the membrane (A) and phase (B) theories of the origin and damage potentials (from Nasonov and Aleksandrov, 1944).

difference of the phase potentials arising in the place of contact of the damaged (or excited) part of the protoplasm and of the liquid electrode with the intact part of the live matter. To the difference in the phase boundary potentials should be added the difference of the diffusion potentials, which arise in the protoplasm as a result of the uneven mobility of the ions. In a medium with a small amount of dissolved electrolytes this potential difference can be quite significant, as follows from the equation of Henderson (1907).

From the point of view of these authors, the RP is not preformed, but arises at the place of damage of the protoplasm owing to the decomposition of the protein-electrolyte complex of live matter. The large amount of bound potassium in the protoplasm (in contrast to sodium and chloride) and the ability of live matter to release it into solution give grounds for supposing that bioelectric potentials occur chiefly as a result of the redistribution of this cation. As a result of the outburst of metabolism at the place of damage or excitation hydrogen ions should also be formed, which is indicated by the

displacement of the pH towards the acid side upon damage (and excitation). Furthermore, phosphates are released in this process. These ions, which differ widely in their mobility, should make the damaged or excited section of the protoplasm very strongly negative. Satisfactory confirmation of the fact that the occurrence of bioelectric potentials is connected with the release of ions may be found in the discovery of Ernst (1928), who showed that before contraction of a muscle its volume decreases, and at the same time an action current is set up. The only explanation that can be found for this phenomenon, so Ernst supposes, is that upon excitation a fresh formation of ions occurs, which leads to electrostriction and the occurrence of bioelectric potentials. This is also supported by the fact that in damaged muscles the fraction of free (quickly exchanging) potassium increases (Troshin, 1960a; Sorokina, 1961a, b).

In the framework of the phase theory the explanation of the excess of the action potential over the resting potential is that more potassium ions are released from the bound state and go into solution in the excited sector of the protoplasm than occurs upon mechanical damage—upon cutting a muscle or nerve fibre, or upon the introduction into the fibre of a glass electrode (Nasonov, 1959a).

Thus, the phase theory of the origin of bioelectric potentials is close to the old alterational theory of Ludwig German (1885); German supposed that the potential difference is formed at the place of alteration of the live substance owing to some chemical processes occurring at the point of damage or excitation of the tissue. The phase theory also has some elements of affinity to another theory which was developed by Chagovets (1896, 1903). According to this theory, resting potentials are set up as a result of the diffusion into a fibre of carbonic acid, and other cations and anions, which form at the place of injury or excitation (see also Chagovets, 1906).

From the point of view of the phase theory of bioelectric potentials, the mechanism of the occurrence of the so-called salt potentials does not differ in principle from the mechanism of the injury and action potentials. If solutions of two different electrolytes in equal concentrations are brought into contact with two points of resting and undamaged protoplasm, a potential difference may be discovered. This potential difference occurs because different ions have different distribution coefficients between the protoplasm and the surrounding aqueous medium. To this PD is added as well a diffusion potential difference, which occurs because the mobility of different ions within the protoplasm should differ.

If the live protoplasm really is a phase of coacervate nature, then it could be expected that the same salt potentials occur in coacervates as are observed in live objects. To check this supposition, I performed an experiment, in which I studied the salt potentials obtained in experiments with frog calf muscles and with a complex coacervate of gelatine and gum arabic (Troshin, 1948b).

In a series of experiments the PD was determined for the chlorides of potassium, rubidium, caesium, ammonium, sodium and lithium. These salts were all used in 0.1 N concentration. The PD was measured in circuits with the coacervate relative to the medium and relative to sodium chloride. The same was also done in circuits without the coacervate (with the medium alone). Parallel measurements were made of the salt potentials in the frog calf muscles. In this case the PD was measured relative to 0.1 N NaCl. The results of these experiments are shown in Table 102.

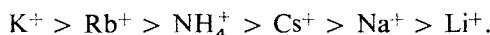
TABLE 102. THE VALUE OF THE SALT BRIDGE POTENTIALS OF COACERVATES AND MUSCLES AND ALSO OF THE DIFFUSION POTENTIALS OF THE MEDIUM. (The plus and minus signs refer to the side of the circuit with the electrolyte in question. Mean values of 3–5 experiments).

Electrolyte	Salt circuits		
	NaCl/medium/ electrolyte	NaCl/coacervate/ electrolyte	NaCl/muscle/ electrolyte
KCl	-11.6	-18.7	-26.7
RbCl	-12.0	-16.6	-16.5
NH <sub>4</sub> Cl	-11.5	-15.8	-13.3
CsCl	-12.5	-14.9	-11.2
NaCl	0	0	0
LiCl	+ 8.7	+ 10.7	+ 6.5

In comparing the rows of figures in Table 102, attention should be directed first of all to the difference between the sequence of the cations in the first case (for the medium) and their disposition in the two latter cases (for the coacervate and muscle). For the medium a purely diffusional series is obtained. The position of each cation in the series is determined by its mobility. This is the so-called lyotropic series of Hofmeister, which reflects the varying capacity of cations for solvation. It is of the following form:



These same cations form a completely different series when they are placed in order of their ability to make the surface of live muscles negative. If the whole business could be reduced to the diffusion of these ions through a membrane determined by the radius of the ions, then we should expect the same series as is shown above. In fact, the following series of cations is obtained for muscle:



The same cation series in order of their ability to make the surface of muscle negative was obtained by Höber (1905, 1908), Seo (1924), Golovina (1948)

and Zander (1959). Netter (1927) obtained the same series for nerve. By comparison with Hofmeister's lyotropic series, we find here inversion of the left part: potassium is displaced to the left, caesium to the right. The coacervate experiments yielded exactly the same results as the muscle experiments.

In the salt circuits with the coacervate the value of the PD obtained was very close to that found in the experiments with muscles. Further, as follows from the table, their magnitude is greater than the diffusion PD's obtained in the circuits with the medium. It may be supposed that the excess of the salt PD of the coacervates over the diffusion PD of the medium is due to the unequal solubility of ions in the coacervate by comparison with that in the aqueous solution and by the varying mobility of the ions within the coacervate.

In experiments with complex coacervates there are obtained not only salt potentials like those observed in muscles, but also concentrational potential differences. Thus, Bungenberg de Jong and Westerkamp (1936) in experiments with a complex coacervate of gelatine and sodium nucleinate obtained a considerable concentrational effect for the chlorides of potassium, sodium, lithium and other electrolytes. The concentrational circuit  $0\cdot1\text{ N KCl}$ —coacervate— $0\cdot01\text{ N KCl}$  gives on the negatively charged coacervate (in the presence of an excess of sodium nucleinate) a PD with a minus sign on the side of the greater salt concentration, and on the positively charged (with an excess of gelatine) the same circuit gives the reverse sign, while on the neutral coacervate  $\text{PD} = 0$  ( $\text{pH}$  everywhere 3.7).

These authors explain their data from the point of view of the theory of Michaelis (1925), who considered the coacervate as a liquid membrane. At the same time they think it possible to interpret their data in the spirit of the theory of Beutner (1920, 1933): Beutner took a negatively charged coacervate to behave like an "acid oil", a positively charged coacervate like a "basic oil", and a neutral coacervate like a "neutral oil".

It seems to me that there is no basis for the view of the coacervate as a liquid membrane when there is so much water in it (about 80 per cent). It is known that concentration and salt potentials were obtained by Michaelis and his colleagues in dried membranes.

The second explanation is probably much nearer the truth, namely, that the concentration effect is connected with the behaviour of the coacervate *qua* phase. This is all the more probable because coacervates can also be characterised as a phase in view of a number of their other physico-chemical properties. Thus, the occurrence of the salt and concentration potentials obtained in experiments with live tissues can be explained without recourse to the assumption of a semi-permeable membrane on the surface of the cells if it is acknowledged that the protoplasm behaves like a phase of coacervate nature.

In recent years the phase theory of bioelectric potentials has been sub-

jected to criticism by a number of workers. The objections to the phase theory reduce basically to the following:

1. The resting potential is preformed in the membrane and does not arise anew. Proof of this is the fact that the RP remains large for a very lengthy period and rises with a jump, greatest in magnitude at the moment of puncture of the cell surface (Kostyuk, 1957; Kurella, 1959a, b, 1960; Sorokina, 1961a).

2. The internal electrical conductivity of the cell is not very different from the conductivity of the surrounding medium. This indicates that the basic mass of the intracellular potassium is in the ionised form and can move freely under the action of electrical forces (Chailakhyan, 1959; Liberman and Chailakhyan, 1959, 1961; Liberman, 1961; Kostyuk, 1960; Sorokina, 1961a and others). Therefore the diffusion potential difference inside the undamaged protoplasm, whose existence is postulated by the phase theory, can be only very small in magnitude, as follows from the formula of Henderson (1907; see also Cole and Moore, 1960).

3. It is considered proven (Hodgkin, 1939, 1958; Katz, 1948, 1952a; Tasaki, 1957) that excitation is propagated along the nerve and muscle fibres by means of local currents owing to the fact that the fibres of excited tissues have cable properties. The surface layer (membrane) has a high resistance, which ensures the distribution of the current for a considerable distance from the point of generation of the electromotive force. The rate of propagation is limited by the resistance of the extracellular medium and the protoplasm through which the capacity of the surface layer is discharged. Upon excitation the resistance of the surface layer falls sharply (Hodgkin, 1951, 1958; Chailakhyan, 1957, 1959, 1960; Liberman and Chailakhyan, 1959, 1961; Liberman, 1961). It is considered that upon excitation an e.m.f. arises on the surface of the fibre, proof of which may be found in the experiments of Baker, Hodgkin and Shaw (1961) on the replacement of axoplasma by solutions.

It must be admitted that the arguments adduced against the phase theory (though they do not touch its basic propositions and are not insuperable for it) make its thorough modernisation essential. We shall discuss below some modern hypotheses which are essentially variants on the phase theory of bioelectric potentials.

*The fixed charge-induction hypothesis.* According to the hypothesis of Ling (1957, 1958, 1960), potassium ions accumulate in cells owing to selective adsorption, which is determined by the effectiveness of the fixed anion charges (see earlier chapter, p. 347). Potassium ions are held in the surface layer of the cell in a high concentration for the same reasons as inside the cell. Therefore, so Ling considers, the resting potential is in the first approximation equal to the potassium balance potential, its magnitude being determined according to Nernst's formula as the potential of a potassium electrode. According to Ling, the RP is determined by the ratio of the con-

centration of adsorbed ions of potassium on the surface of the cell to the concentration of these ions in the surrounding medium. Ling uses this to explain the independence of the RP of the introduction into the cell of univalent ions and the almost instantaneous drop of the RP to a constant level, found from Nernst's equation, upon immersion of the nerve or muscle fibre into Ringer's solution with elevated potassium ion content. It is interesting that Aschheim (1959, 1960, 1961), starting from the size of the ion currents, calculated that the formation of the potential difference occurs on the surface of the cell upon the adsorption into a single layer of potassium ions in rest and sodium ions in excitation.

According to Ling's hypothesis, the excitation of nerve or muscle fibre is accompanied by a definite kind of change in the protein molecules of the cell surface, leading, thanks to the induction effect, to a change in the effective fixed anion charge: this charge loses its adsorptional affinity for potassium and acquires an affinity for sodium ions. Consequently an action potential is set up, whose magnitude is, in the first approximation, determined by Nernst's formula as the potential of a sodium electrode (equation 4).

A similar view of the nature of resting and action potentials was expressed by Ungar (1959). In a series of works Ungar and his colleagues showed that, upon excitation, proteins isolated from cells do indeed acquire an affinity for sodium and lose their affinity for potassium (Ungar, Aschheim, Psychoyos and Romano, 1957; Ungar and Romano, 1958, 1959; Ungar, 1959, 1961a, b; Ungar and Kadis, 1959). These facts can be considered as a serious argument in favour of Ling's hypothesis.

In his work Ling suggests no scheme for measuring the resting and action potentials which, in his opinion, occur on the boundary between cell and medium. In nature these potentials are akin to the zeta-potential. It is hardly likely that a normal microelectrode technique will be adequate for picking up such a potential. In any case, if the accumulation of potassium in cells takes place owing to selective adsorption over the whole thickness of the protoplasm, then the introduction into a cell immersed in Ringer's solution of a microelectrode also filled with Ringer's solution should lead to the discovery of a zero RP. In fact, what is measured in these conditions is a RP of constant magnitude, independent of whether the microelectrode is filled with Ringer's solution or a solution of potassium chloride of various concentrations (Nastuk and Hodgkin, 1950; Cole and Moore, 1960, etc.).

In an attempt to get round this difficulty in Ling's hypothesis, Kurella (1959a, b, 1960, 1961a, b) suggested that there is on the surface of a cell a membrane with phase properties different from those of the protoplasm itself. In this case, he considers, the magnitude of the RP should not depend on the electrolyte composition of the microelectrode introduced into the cell. In support of his hypothesis he performed a series of experiments with ion-exchange resins, which confirmed his hypothesis.

*The phase-membrane hypothesis.* Very recently Liberman (1961; see also

Liberman and Chailakhyan, 1961) has introduced extensive changes into the phase theory of bioelectric potentials. He starts from the fact that the protoplasm has phase properties (a "non-aqueous medium"), but, in contrast to the theory of biopotentials developed by Nasonov and Aleksandrov, he assumes the presence in the cell of a considerable amount of potassium and sodium in the free (ionised) state and also a high resistance in the surface layer (protoplasmic membrane) and a change in this resistance upon excitation. This new hypothesis was called the "phase-membrane" (Liberman, 1961). Its essence is the following.

The observed RP and AP when there is a considerable proportion of potassium in the free state in the cell can occur if the protoplasm has the properties of a "non-aqueous phase" in the sense that the energy of the ions upon transition from the aqueous solution into the protoplasm changes not only as a result of the existence between them of a potential difference. Ions passing from the aqueous medium into the protoplasm reduce their hydrate shell. The mobility of the ions in the protoplasm can therefore be the same as, or even higher than, in water. The energy of all the ions in the protoplasm-phase will be greater than in the aqueous solution, but their concentration will be lower than in the surrounding aqueous medium. A large number of such solutions is known between which such an ion distribution is observed. However, the protoplasm differs from them in an important property: anions cannot penetrate it, because it contains bound (fixed) anions — protein anions or polyelectrolytes which cannot go out into the surrounding solution. Consequently, only those cations are released whose energy in passing through the water — protoplasm boundary changes more strongly, their place being taken by the cations which are more weakly ejected. The stronger changes take place in the energy of calcium, lithium and sodium, which have a large hydrate shell, the ions remaining inside being of potassium, rubidium and caesium whose hydrate shells are of smaller radius. The electric field of the protein (fixed) anions also ejects mobile anions, including chloride ions. A similar hypothesis of the redistribution of inorganic ions between cells and medium was recently put forward by Norman *et al.* (1961).

The ratio of the concentrations of various ions inside and outside a cell should be determined by the energy barriers erected by the chemical PD of the ions and the electric field PD caused by the protoplasm anions. To describe the experimentally observed ion distribution in the resting fibre of excitable tissues, these barriers ( $V$ ) on the protoplasm-medium boundary should have the following values (in volts):  $V_{\text{Ca}} = 0.2$ ;  $V_{\text{Na}} = 0.057$ ;  $V_{\text{Cl}} = 0.2$ ;  $V_{\text{K}} = -0.093$ . Calculations show that such barriers are obtained upon transition from an aqueous solution with a dielectric constant  $\epsilon = 80$  into a protoplasm with  $\epsilon = 50-60$  (Liberman, 1961). In the literature, as we shall see below, there are data which indicate a low value of  $\epsilon$  for the protoplasm of resting cells. This is also supported by the data quoted in the preced-

ing chapters which point to the lowered ability of substances to be dissolved in resting and undamaged cells.

These energy barriers make it possible to explain the value of the RP which is found experimentally. In fact, if one part of a fibre is immersed in Ringer's solution and the other in a solution of potassium chloride, then the greater permeability of the protoplasm for potassium results in the carriage through the protoplasm of these ions together with their positive charge. The resulting potential difference can be measured and calculated by means of an equation deduced by Liberman (1961) for a membrane with selective ionic permeability.

The value of the RP taken off by an intracellular electrode is also described by this formula if it is recognised that the electrode destroys the phase round about itself (that is, changes the energy of the ions), changing the protoplasm at the point of introduction of the electrode into an aqueous solution with a high potassium ion content, as foreseen in the phase theory of Nasonov and Aleksandrov. In this case the value of the RP should be independent of the electrolyte concentration in the microelectrode because what is in contact with the intact part of the protoplasm is not the electrode, but the destroyed protoplasm.

According to the phase-membrane hypothesis excitation results in the destruction of the surface layer and part of the protoplasm in about the same way as the protoplasm is destroyed by the electrodes introduced into cells. In this case the potential difference between two aqueous solutions over the surface and under the surface of the cell will, independently of the ionic composition, be close to zero and the RP will fall to zero. The drop in the RP and its subsequent re-establishment is in fact the action potential (AP). The sodium concentration in the external solution will have practically no effect on the value of the AP. This is the state of affairs observed in crab muscle fibres.

In the nerve and muscle fibres of vertebrate and cephalopod molluscs excitation is observed to result in a change in sign of the potential, the magnitude of this inverse potential (excess of the AP over the RP) depending on the sodium ion concentration in the medium. To describe this effect it is assumed that there is in such objects a passive membrane which is always selectively permeable for sodium. This assumption agrees with the value of the capacity of the surface layer, which in muscle fibres of crustacea is  $20-30 \mu\text{F}/\text{cm}^2$ , and in nerve and muscle fibres of vertebrates is  $1-10 \mu\text{F}/\text{cm}^2$ . Upon excitation the layer of the protoplasm under the passive membrane is destroyed and the sodium ions rush into the cell. In this case the amplitude of the inverse potential will be described by the same formula as in the sodium hypothesis (formula 4). If the re-establishment of the broken surface layer of the protoplasm is for any reason delayed, equilibrium is rapidly established between the external solution and the damaged layer of the protoplasm, the sodium ion concentrations equalise and the difference between

them falls to zero. Here the same picture is obtained as that observed during extended action potentials (EAP).

Bivalent calcium ions or analogous strontium and barium ions may play an important role in the reactions which give rise to the AP. To explain the change in sign of the potential upon excitation and the observed magnitude of the AP in solutions of strontium or barium chloride, this reaction can be seen as the displacement of bound ions of these metals into the depths of the fibre along a chain of protein polyelectrolyte; the bivalent ions could give rise on the surface to a film which was selectively permeable for the corresponding bivalent ions from the external solution, because the following barium or strontium ion can occupy the place of the first ion, driving the whole chain of bound ions into the depths of the fibre. This film will be less permeable for potassium ions because all or the greater part of the anions will be bound with the bivalent ions in the surface layer. This surface layer with selective permeability for strontium or barium ions would create between the external solution and the inner part of the fibre a potential difference which would, in the first approximation, be proportional to the logarithm of the external concentration of bivalent ions in the medium, as is in fact observed experimentally. It is not impossible that a similar reaction with sodium ions may give rise to a layer with selective permeability for sodium ions.

The quantitative side of the phase-membrane hypothesis about the origin of bioelectric potentials has been only very weakly developed; in accounting for the results obtained in recent years, it experiences no less difficulties than the modern membrane theory.

### C. Bioelectric potentials and cellular metabolism

Bioelectric potentials are connected with the liberation by the live protoplasm of energy. This energy is obtained at the expense of metabolism. The idea that metabolic energy is the source of electrical energy in live tissues originates from Sechenov (1862, 1866). To-day this concept of Sechenov's has won widespread recognition.

In recent years many works have been published in which an attempt is made to establish the dependence of the generation of an electric potential by a tissue on the metabolic processes in it. Among these are the publications of Muzheyev and Bordzyko (1933), Mikhel'son (1935), Karayev (1937), Kostoyants (1938, 1939, 1944), a series of works by Lebedinskii and his colleagues (Lebedinskii, 1939; Lebedinskii, Gramenitskii *et al.*, 1947; Mozzhukhin, 1948a, b, 1950a, b; Lebedinskii and Mozzhukhin, 1950; Ilyinskii, 1950; Kon-torovich, 1950; Tovbi, 1950; Yaroslavskaya, 1950), the meticulous study of Lorento do No (1947), a series of papers by Sorokina (1961a, b) and others.

Muzheyev and Bordzyko, and subsequently Mikhel'son, established that poisoning muscles with sodium cyanide and monooiodoacetic acid results in

a fall in the resting potential. At the same time the muscles are observed to stiffen. Mikhel'son draws from this the, in my view, correct conclusion that the resting potential falls as a result of deep destruction of the tissue structure.

Lebedinskii and his colleagues, coming out against the basic propositions of the membrane and phase theories of bioelectric currents, assert that these theories appear to neglect the role of metabolism in the generation of electrical energy by live matter. Their point of view can be summarised as follows: the generation of electrical energy is due to metabolic reactions in the undamaged or unexcited part of tissue, which continuously maintain the polarisation of the protoplasm at a definite level. However, this point of view does not, in fact, contradict either the phase or the modern membrane theory of bioelectric potentials, because these theories of the origin of bioelectric potentials are essentially theories of the transformation of metabolic energy (chemical energy) into electrical energy.

According to the membrane theory, it is at the expense of metabolic energy that the definite structure of the cell membrane is maintained and the difference in ion concentrations on either side of this formation is ensured (Rubinstein, 1944, 1949b; Vorontsov, 1947; Grundfest, 1947; Hodgkin, 1951, 1958, and others).

From the point of view of the phase theory of bioelectric potentials the energy of metabolism is used in the synthesis of a protein-electrolyte complex which easily decomposes under the action of irritants, this being in fact the origin of the PD. Metabolic energy maintains on a definite level the phase properties of the protoplasm which affect the magnitude of these potential differences (Nasonov, 1939, 1949a, 1959a; Nasonov and Aleksandrov, 1943, 1944).

The modern membrane theory explains the asymmetry of the distribution of inorganic ions (which is actually the cause of the differences in bioelectric potentials) by the existence of membrane ion pumps, which work at the expense of the energy of decomposition of high energy phosphorus compounds (for a review of the literature see: Hodgkin, 1958; Keynes, 1961). However, according to Grundfest (1955), the unequal distribution of ions on either side of the cell membrane is not the cause of the RP. He connects the origin of the RP directly with the activity of the "sodium pump", —the forces driving the sodium ions from a cell are greater than the forces which make for their movement in the opposite direction. The asymmetry in the oppositely directed currents of sodium ions also leads to the occurrence of potential differences balancing the movement of these ions in either direction.

Lorento do No (1947) connects the origin of these potential differences directly with the chemical processes which go on in the cell membrane. He observed that frog nerve immersed in an oxygen-free medium suffers slow depolarisation which lasts several hours. When the nerve is returned to a normal atmosphere, the resting potential in a few minutes becomes 15 to

20 per cent greater than originally. The same also occurs in a medium free of potassium ions. These facts, as the author points out, are in serious conflict with the membrane theory. According to Lorento do No, the surface of a cell (membrane) has a double electric layer, maintained by chemical forces, which continuously hold apart the charged particles of opposite sign. According to this hypothesis, the value of the RP is determined by the equilibrium between the Coulomb forces of attraction and the chemical forces acting on the charged particles. The membrane is seen as a field of electrical and chemical forces which is created by oxidising metabolism. From this point of view, the heterogeneity of the inorganic ion distribution between cell and medium is not the cause, but a consequence of the existence of the membrane potential.

Ling (1952, 1955), in a discussion of a large number of studies of the effect of metabolic inhibitors on the value of the RP, came to the conclusion that metabolic reactions affect the generation of bioelectric potentials indirectly, by maintaining a definite protein structure of the protoplasm, which ensures the selective accumulation of potassium. The same conclusion has recently been reached by Sorokina (1961a, b). This viewpoint does not differ from the views on this question of Nasonov and Aleksandrov, the authors of the phase theory of biopotentials (Nasonov, 1939, 1949a, 1959a; Nasonov and Aleksandrov, 1943, 1944).

Different in principle is the origin of bioelectric potentials suggested in the theory of Lund (1928). He supposes that the direct cause of biopotential differences is the difference in the redox potentials inside and outside a cell which occur as a result of cellular metabolism. This theory met with a number of objections (see Rubinshtein, 1947; Nasonov, 1949a, 1959a; Arshavskii, 1960) in the sense that a redox potential can be transformed directly into an electrical potential difference and can be taken off by liquid electrodes only in the presence of metal electrodes, of which there are none in a cell.

However, Kometiani (1949, 1956, 1961a, b) supposes that bioelectric potentials are at least partially due to redox potentials. He considers that oxidising-reducing reactions in a cell facilitate the direct accumulation in the protoplasm of potassium in exchange for hydrogen ions. Further, the oxidising-reducing reactions in a cell are spatially separated by the cell structures which are the cause of the varying mobility of ions. In view of this the directed displacement of electrons should lead to polarisation of the separated surfaces as a result of the differing mobility of the ions. This leads to an orientation of the ions such that a potential gradient is established which can be measured by liquid electrodes.

Against the view that chemical energy is somehow directly converted in cells into electrical energy is the fact that the RP is not significantly temperature dependent (Bernstein, 1902; Hodgkin and Katz, 1949b; Nastuk and Hodgkin, 1950; Fatt and Katz, 1953; Arshavskii, 1958; Veprintsev, 1959;

Veprintsev and Antonov, 1959; Burlakova, Veprintsev and Rass, 1959; for a review of the literature see Arshavskii, 1960).

## 2. The Electrical Conductivity of Cells; Impedance

It has been established by numerous investigators that the electrical conductivity of live cells is very low—many times lower than the conductivity of the surrounding medium.

The specific resistivity of large plant cells (*Valonia*, *Nitella*, *Laminaria*), nerve conductors, muscles, erythrocyte suspensions, yeast cells and other live cells varies within the limits of several hundreds to several thousands of ohms per  $\text{cm}^2$  of cell surface.

The electrical conductivity of excited and damaged cells is considerably elevated. Dead cells as a rule have the same conductivity as the surrounding medium (Osterhout, 1936a; Cole, 1940; Duyff, 1942; Rubinshtein, 1947 etc.).

In view of the fact that cells are conductors of the second kind, their electrical conductivity for constant and low frequency alternating currents should be determined principally by the concentration in the cell of free ions (basically, inorganic ions), their mobility and the ability to diffuse into the cell and out of the cell into the surrounding medium.

The membrane theory explains the low electrical conductivity of cells by the presence on the surface of the protoplasm of a membrane which has the properties of a dielectric and prevents the passage of ions. Upon excitation and damage of the cells the permeability of the membrane for ions increases, resulting, so this theory asserts, in an increase in the electrical conductivity of the cells. In fact, a number of authors using the micro-electrode technique and other methods have determined the specific conductivity of the protoplasm of muscle and nerve fibres of various animals: it turned out that this is very high—only 1·5–3 times lower than the conductivity of the surrounding medium. The specific resistivity of the boundary layer of the fibres (cell membranes) stand out in virtue of its high resistance (hundreds and thousands of  $\Omega \text{cm}^2$ ) and its large capacity ( $1\text{--}40 \mu\text{F}/\text{cm}^2$ ). These data make it possible to consider muscle and nerve fibres as formations having the properties of cables (Hodgkin and Rushton, 1946; Katz, 1948; Weidmann, 1951, 1952; Fatt and Katz, 1951, 1953; del Castillo and Machne, 1953; Boyd and Martin, 1959; Cole and Moore, 1960 and others).

The explanation given for this phenomenon by the membrane theory is, however, in contradiction to the large amount of facts discussed above. As we have seen, it has been firmly established by the method of labelled atoms and other techniques that in the vast majority of cases inorganic ions can pass into a cell and from the cell into the surrounding medium at a great rate. And the very fact that cells are conductors of electrical current indicates that cells are permeable for inorganic ions (Nasonov, 1939, 1949a; Cole, 1940; Heilbrunn, 1952).

In our discussion of the state of salts in cells on the basis of the available experimental data we came to the conclusion that part of the inorganic substances present in the protoplasm is there in the bound form. Further, the mobility of the free ions in the protoplasm is very evidently considerably lower than their mobility in the surrounding aqueous solution, as was shown by Yefimov and others (Yefimov, 1952, pp. 155-6). This also leads to lowered electrical conductivity of the cells.

In the excited state or upon damage to the cells, as we have seen, bound mineral substances are liberated and pass into the dissolved, ionised form. It is intelligible that this should lead to increased electrical conductivity of the cells, as is in fact actually observed.

Alternating, not direct, current is used to measure the electrical conductivity of cells so as to avoid polarisation on the electrodes. Further, the passing of a direct electric current through a cell is often observed to result in the disintegration of the protoplasm, just as happens in the case of complex coacervates. This leads to damage of the cells and sharp increase in their conductivity.

It is known that the resistance of solutions is independent of the frequency of the alternating current (provided that the frequency is not greater than  $10^8$  c/s); whereas the resistance of live cells is frequency dependent. At frequencies from 0 to  $2 \times 10^4$  c/s the resistance of a live cell is at its maximum and is almost independent of frequency; at higher frequencies it falls sharply and reaches a constant level at frequencies of  $10^6$ - $10^8$  c/s. In this frequency band the resistance of a live cell is at its absolute minimum and is almost independent of the frequency of the alternating current.

These facts indicate that a live cell has not only ohmic resistance, which is independent of the a.c. frequency, but also capacitative (or reactive), which is frequency dependent. The total resistance of cells, which is made up of ohmic ( $R$ ) and capacitative ( $X$ ) components, is called the impedance ( $Z$ ). The literature on this subject is very extensive (see reviews: Osterhout, 1936a; Tarusov, 1939; Yun'yev, 1939; Cole, 1940; Duyff, 1942; Rubinshtein, 1947, 1949 b; Hodgkin, 1951).

To obtain an understanding of the essence of cell impedance so-called "equivalent schemes", consisting of capacity and resistances, were devised. The simplest of all the suggested equivalent schemes consists of a capacity with a resistance connected in parallel. The impedance of such an electrical system is:

$$Z = \sqrt{[R^2 + (1/\omega^2 C^2)]}$$

where  $R$  is the ohmic resistance,  $C$  the capacity,  $\omega$  the angular frequency, related to the number of alternations of current per second ( $n$ ) by the formula  $\omega = 2\pi n$ .

It can be seen from this equation that for an infinitely large value of  $\omega$  the capacitative resistance ( $X = 1/\omega C$ ) tends to zero, and in this case the impedance of the system will be a minimum equal to the ohmic resistance  $R$ .

Similarly, when  $\omega = 0$ , the impedance will be also purely ohmic, because a constant current does not pass through a capacitance.

In view of the fact that this scheme is far from reflecting the changes in the impedance of cells measured at various frequencies, various workers have suggested other "equivalent electrical schemes" consisting of a capacitance with series and parallel connected resistances. However, even these more complex schemes do not give a correct picture of the electrical properties of cells (Rubinshtein, 1947).

The supporters of the membrane theory think that the capacity of a cell ( $C$ ) is the polarisational capacity of the cell membrane which fulfils the role of the covering of the condenser and serves as a barrier against the passage of ions.

According to this theory, the capacity of a cell is determined by the area of the cell membrane, its thickness and dielectric constant. By measuring the capacity of a cell and the area of its surface, assuming that the membrane consists of lipids whose dielectric constant is known, the thickness of the cell membrane can be calculated (Fricke, 1925; Danielli, 1935, etc.). When the frequency is increased the electric current as it were jumps across the membrane, as a result of which the capacitative resistance of the cell-condenser falls. The ohmic resistance of a cell ( $R$ ) is composed of the resistance of the electrolyte solutions in the cell and outside and the ohmic resistance of the membrane, which is an imperfect insulator, because ions pass through it though with great difficulty (so-called "capacity leakage" or "condenser loss"). Excited and damaged cells have a lower impedance than resting and undamaged cells. On cell death the ohmic resistance falls sharply and the capacitative term vanishes\*.

The membrane theory explains these phenomena by an increase in the permeability of the membranes for ions.

In view of the fact that the ohmic resistance decreases with increasing frequency of the a.c. current and in the limit falls to a small value, the electrical conductivity of cells measured at high frequencies (of the order of  $10^6$ - $10^7$  c/s) characterises the so-called "internal conductivity" of cells, whose considerable magnitude would appear to indicate the presence in the protoplasm of a large amount of free (ionised) electrolytes.

It is known that Höber (1910, 1912, 1913) was the first to build the theoretical foundations and make practical measurements of the "internal conductivity" of cells. He compared with one another the low frequency and the high frequency ( $10^6$ - $10^7$  c/s) electrical conductivity of pure solutions of sodium chloride with the conductivity of centrifuged and washed cattle and pig erythrocytes (the washing fluid being an isotonic sugar solution) and of

\* Tarusov (1938, 1941) and Burlakova (1959) have shown that cells fixed with formalin have a considerable and stable impedance. The impedance is preserved also upon irreversible damage of the cells caused by the action on the tissue of solutions of certain electrolytes and other factors (Maslov and Sverdlov, 1950).

isolated frog muscles immersed in a sugar solution. It turned out that the "internal" (high frequency) conductivity of cells is 5–10 times greater than the low frequency, and that the "internal conductivity" of cells is equal to the conductivity of 0·3–0·4 per cent solutions of sodium chloride. Haemolysis of the erythrocytes caused by the addition of saponin raised the low frequency conductivity to the high frequency level.

On the basis of these data Höber concluded that a considerable part of the inorganic substances is present in cells in the free and ionised state and that the different inorganic composition of the cell and medium, and also the impedance, is maintained by a membrane impermeable for ions which is destroyed upon cell damage.

Numerous investigators in experiments with a very wide range of plant and animal cells have obtained similar data. However, the explanation of these results suggested by Höber is contradictory to other facts about the permeability of cells for salts and the electrical properties of cells in their various functional states. Thus, it follows from the above mentioned experiments of Höber that the "internal electrical conductivity" of cells is about half the conductivity of their balanced saline solution (surrounding medium). If it is recognised that the high frequency conductivity of cells is ensured only by the presence of free ions in the protoplasm, then it must be assumed that about half the inorganic substances are present in the cell in the unionised state and do not participate in the conduction of electrical current. According to the data of McClendon (1927), the high frequency conductivity of erythrocytes corresponds to the conductivity of a 0·1 per cent solution of sodium chloride.

Cole and Cole (1936) discovered that the "internal electrical conductivity" of unfertilised eggs of the starfish *Asterias forbesi* is 4–7 times lower than the conductivity of sea water. This already indicates that the dissolved part of the electrolytes in the protoplasm is many times less than the total concentration of inorganic substances in the cell and in the surrounding medium. Thus, these data confirm the conclusion that part of the inorganic material in the protoplasm is in the bound and unionised state.

In this connection great interest attaches to the work of Pauly (1959). Pauly showed that the internal electrical conductivity of the erythrocytes of humans and various kinds of animals is half the electrical conductivity calculated on the basis of the electrolyte concentration in these cells. The author supposes that part of the ions in the cells is in the bound state and that their mobility is diminished. It was also shown by this author that the dielectric constant of the contents of cells is sharply lowered by comparison with the aqueous solution of the surrounding medium. These interesting results of Pauly's are shown in Table 103.

It has also been shown in Ernst's laboratory (see Ernst, 1958) that the so-called "internal electrical conductivity" of frog muscle fibres is several times lower than the conductivity of Ringer's solution. On the basis of these and

other data he comes to the conclusion that a considerable part of the cell potassium is in the bound state. In support of this he produces the following arguments: (1) potassium is selectively accumulated by muscles out of solution; (2) the process of the accumulation of potassium by cells does not require the expenditure of energy; (3) potassium is retained by muscle at a temperature of 0°; (4) on sedimentation of the muscle proteins out of a homogenate of frozen muscles the greater part of the potassium goes out

TABLE 103. THE INTERNAL SPECIFIC RESISTANCE ( $\rho$ ) AND ELECTRICAL CONDUCTIVITY ( $\kappa$ ) MEASURED AT 90 mc/s, AND THE DIELECTRIC CONSTANT ( $\epsilon$ ) MEASURED AT 250 mc/s, OF THE ERYTHROCYTES OF HUMANS AND A NUMBER OF ANIMALS AT A TEMPERATURE OF 25°

(from Pauly, 1959).

Animal	$\rho$	$\kappa$	$\epsilon$
Human	193	5.18	50.1
Bull	230	4.35	51.3
Sheep	228	4.39	50.4
Dog	216	4.63	50.9
Cat	190	5.26	53.1
Rabbit	170	5.89	55.1
Chicken	204	4.90	52.1

into the sediment; (5) the concentration of potassium in certain structures of muscle is considerably higher than the average potassium concentration of the whole muscle; (6) measurement of the activity of potassium in muscles indicates that it is about 0.5.

Further, a number of authors (see Curtis, 1937; Tarusov and Burlakova, 1939; Cole, 1940 and others) have discovered that erythrocytes haemolysed with water have a considerable impedance. Tarusov and Burlakova (1939) made a detailed study of the low and high frequency electrical conductivity of rabbit erythrocytes, both undamaged and haemolysed with water and saponin, and also determined the quantitative change of the potassium content of such cells. They discovered that the low frequency conductivity of undamaged and water-haemolysed cells is many times lower than the high frequency conductivity, but that the low frequency conductivity of saponin-haemolysed erythrocytes is the same as the high frequency. It was also found that the water-haemolysed erythrocytes do not lose potassium, whereas the saponin-haemolysed cells do release potassium. On the basis of these facts the authors come to the conclusion that the impedance of cells is tied up with the presence in them of potassium, that potassium is unquestionably present in cells bound with the proteins and that this bond is destroyed when the cells are damaged by saponin (upon stromatolysis). The same conclusions, contradictory to the basic propositions of the membrane theory, are also reached by Trincher (1939, 1958), in his study of the change in impedance on

warming of a suspension of erythrocytes to temperatures above body temperature. In the opinion of this author, it is not possible to explain the impedance of cells by means of a membrane mechanism.

Very interesting results were obtained by Maslov and Sverdlov (1950) in their comparison of the change in impedance and polarisation potential (potential differences of salt currents and injury currents) in isolated frog sartorius muscles when these were treated with isotonic solutions and other agents. If the magnitude of the impedance and the polarisational potentials are determined by the properties of a cell membrane (selective permeability for ions), then the change in impedance when different kinds of treatment are applied to the tissue should follow a single-value and parallel change in the polarisational potential. The numerous experiments of these workers show that nothing of the kind occurs. The change in the frequency coefficient of impedance (the ratio of the resistance of the muscles at  $10^4$  c/s and their resistance at  $10^6$  c/s) and the polarisational changes of the muscles in solutions of various salts follow completely different paths. Solutions of potassium and calcium chloride make the muscle negative, while the frequency coefficient in the first case rises and in the second falls. On the other hand, potassium and magnesium chlorides change the frequency coefficient in one direction but have opposite effects on the bioelectric potential difference.

In a series of papers Aladzhalova (1949, 1950) and Aladzhalova and Mertsalova (1954), studying the changes in the ohmic and capacitative resistances of various striated and smooth muscles in relation to their functional state, also obtained a vast amount of material which is in poor agreement with the membrane explanation of the electrical properties of cells.

Thus, the experimental data on the permeability of cells for ions, the state of inorganic substances in the protoplasm and the electrical properties of cells in their various functional states compel us to acknowledge that the high ohmic and capacitative resistance of live cells cannot be explained only by the presence on the surface of cells of a membrane whose properties are responsible for all these phenomena.

The high ohmic resistance of cells can be explained, as has already been remarked, by the fact that the inorganic substances in the protoplasm are partially in the bound state, and by the fact that the mobility of ions in live matter is less than in a homogeneous aqueous solution. To explain the capacitative resistance of cells it is completely unnecessary to have recourse to the assumption of the presence on the surface of cells of a membrane with the properties of an imperfect dielectric with selective permeability for ions. In the literature the suggestion has been made more than once that the existence of a capacitative resistance and its change are possibly not connected with the permeability of the membrane for ions (Curtis, 1937; Tarusov, 1939, 1954; Aladzhalova, 1950 and others).

There are weighty arguments in favour of the opinion that the capacitative

resistance of cells is due to the live matter as a whole as a heterogeneous colloidal system. According to Tarusov (1939) the capacitative resistance of a cell should occur already by virtue of the fact that the disperse medium and disperse phase of the protoplasm have different electrical conductivity and different dielectric constants. This phenomenon is also observed when an electric current is passed through a homogeneous medium in which there is emulsified or suspended some other substance which differs in its electrical properties from the medium. In this case the change in the polarisational capacity of a cell upon excitation and damage should be connected with the change in the electrical conductivity and dielectric constant of the disperse phase and disperse medium of the protoplasm. Aladzhalova (1950) connects the existence of the capacitative resistance of cells with a double electric layer on the micelle-medium boundary, and the change in these capacities under the action of various treatments on the protoplasm and in dependence on the functional state of the cells with changes in this double electric layer. According to her ideas, the impedance measured at various a.c. frequencies characterises the different aspects of the electrical structure of the protoplasm. The conductivity of cells for 1.f. current (of the order of  $10^3\text{--}5\cdot10^4$  c/s) is affected principally by the ion concentration on the polarisable boundaries (micelle-medium), while in the h.f. range ( $10^5\text{--}10^6$  c/s), the dominating influence on impedance is the polarisation of the orientation. At these frequencies the conductivity of the protoplasm is due to the orientation of the polar molecules in the direction of the electric field formed between the applied electrodes and the quantity of free charges. In this frequency range, so the author supposes, the impedance depends on the dipole moment of the molecules of the live matter, on the electrostatic interaction between them and so on.

### 3. Conclusions

1. It may be supposed that bioelectric potentials are of phase nature. Upon excitation and damage of cells decomposition of the protein-electrolyte complex occurs. The electrolytes passing into the dissolved state, on contact with the intact or resting part of the protoplasm, form a phase boundary potential. The phase boundary potential also arises when a liquid electrode comes into contact with the intact part of the live matter.
2. Injury (rest) and excitation potentials can be seen as the difference of the phase boundary potentials arising at the point of contact of the injured or excited part of the protoplasm and the liquid electrode with the intact (resting) part of the protoplasm. It is possible that in addition to the phase boundary potentials there are also diffusion potential differences in the protoplasm.

3. There are grounds for supposing that the phenomenon of impedance is connected with the presence in the protoplasm of capacitative resistances caused by the fact that live matter is a polyphase colloidal system whose

components (micelles of colloids) differ from one another and from the disperse medium in their electrical characteristics (conductivity, dielectric constant, etc.). Not only the ions present in the protoplasm, but also dipoles of organic molecules forming undissociated and dissociated complexes with the inorganic substances may participate in the conduction of high frequency electric current.

4. There is considerable foundation for the idea that bioelectric potentials are ionic in nature. In explaining the available data the membrane and phase theories of bioelectric phenomena meet considerable difficulties. Both these theories require fundamental modernisation.

## CHAPTER XII

### The Protective Action of Non-Electrolytes against Damage of Live Matter Caused By Dilute Saline Media

ONE weighty argument in favour of the membrane theory has till now been considered to be the fact that part of the sodium chloride from the medium surrounding a cell can be replaced by some indifferent non-electrolyte without visible damage to the live matter.

As is well known, Overton (1902b) immersed isolated frog muscles in a physiological solution in which the sodium chloride concentration had been diluted about 7 times and the amount of salt required to make the solution isotonic had been replaced by sugar.

In such a solution the muscles did not differ in respect of their external appearance, excitability and length of life from muscles placed in ordinary physiological solution. In Overton's opinion, the essence of this "protective effect" consists in the concentration of mineral substances inside muscle fibres placed in such a medium remaining unchanged because the sugar and sodium chloride ions, so he considered, do not penetrate inside the fibres or the mineral ions inside them move out.

This viewpoint of Overton's is shared even to-day by many of the supporters of the membrane theory. Thus, Rubinshtein (1947) writes the following on this question: "A considerable part of the sodium chloride in blood serum or Ringer's solution is in fact of osmotic significance only and can be replaced by an indifferent electrolyte" (p. 189).

It is obvious that such a viewpoint is erroneous; the protective effect of non-electrolytes against the action upon the cell of dilute saline solutions cannot be reduced to an osmotic effect, because the assertion of the impermeability of live cells for inorganic ions and non-electrolytes has proved to be wrong: a very wide variety of non-electrolytes, including sugar, and also ions of sodium, lithium, potassium, chloride and phosphate, can, as we have seen, pass into live cells. Muscle cells are also permeable for choline (Renkin, 1961), vital dyes etc. A change in the concentration of these substances in the medium leads to a change in the amount of them in the protoplasm and both their passage into cells and release from them can be observed in conditions when the protoplasmic water content remains at its initial level.

Consequently, the physiological effect of the protection observed when part of the salt in the medium is replaced by a non-electrolyte cannot be satisfactorily explained within the framework of the membrane theory, so that we must seek some other explanation of this interesting fact.

To explain the essence of this phenomenon I have studied the effect on the life of isolated frog muscles of dilute Ringer's solutions both with and without electrolytes (Troshin, 1939). The fundamental results from these experiments are given in Figs. 124 and 125.

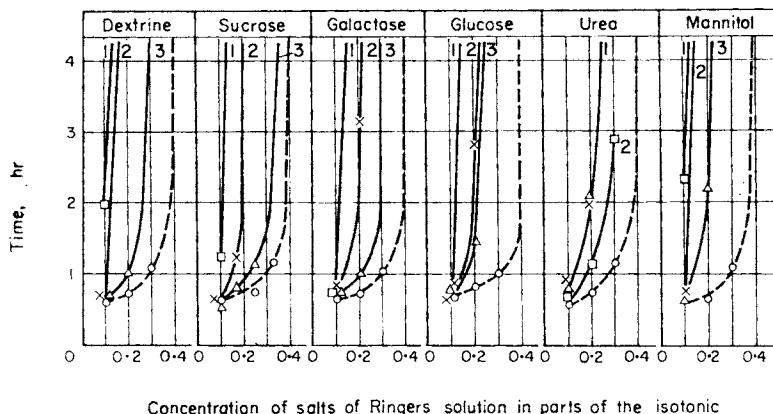


FIG. 124. The dependence of the life of muscle on the salts' concentration in Ringer's solution (R) with and without the presence of non-electrolytes.

The solid lines correspond to the solutions: dextrine—1 — 0·038 M + R, 2 — 0·019 M + R, 3 — 0·009 M + R; sucrose—1 — 0·142 M + R, 2 — 0·072 M + R, 3 — 0·036 M + R; galactose—1 — 0·278 M + R, 2 — 0·139 M + R, 3 — 0·069 M + R; glucose—1 — 0·278 M + R, 2 — 0·139 M + R, 3 — 0·069 M + R; urea—1 — 0·839 M + R and 0·417 M + R, 2 — 0·209 M + R; mannitol — 1 — 0·862 M + R, 2 — 0·431 M + R, 3 — 0·216 M + R. The broken lines correspond to Ringer's solution (control).

The dilution of Ringer's solution (1·0 R) to 0·4 R has no essential effect on the life of the muscles—they maintain their excitability for a long time. However, in solutions diluted to 0·1–0·3 R muscles live 30–40 min only: they swell strongly and go cloudy. The same phenomenon was later observed by Gavrilova (1948).

As the continuous lines show, the non-electrolytes added to the dilute saline solutions can be divided into two groups by the effect they produce. One group of substances protects the muscles to a greater or lesser degree from the destructive action of saline hypotony; the other, on the contrary, aggravates this action. It proved that the non-electrolytes of the first group do not have equal protective power: to produce the same protective effect different concentrations of these substances were required.

In order of their protective power the non-electrolytes of this group can be placed in the following series:

dextrine > sucrose > galactose, glucose > urea >  
> mannitol > glycerol > glycol.

It is interesting to note that the substances in this series, with the exception of urea, are in order of molecular weight.

Glycol shows the least protective effect. In its action it is as it were a transition substance to the second group, which includes acetone, alcohol and ether. These only reinforce the damaging effect of the dilute saline solutions.

Independently of whether there is an osmotically active membrane on the surface of cells or not, when live cells are immersed in hypotonic salt solutions we shall always have a reduction of their concentration inside the cell. Our attention should, therefore, be directed first to the effects which the cell globulins can suffer in this process. These proteins, as is known, coagulate with an insufficient salt content and fall out of solution in the form of a solid precipitate. Kamnev (1936) in a study of cell paraneurosis caused by salt hypotony came to the conclusion that depriving the protoplasm of its minimum of salts—the dispersion agents of the micelle proteins—reduces the dispersion of the cell colloids, as a result of which the whole complex of the paraneurotic state of the protoplasm arises.

Since the protective effect produced by non-electrolytes cannot be explained from the osmotic point of view because of their ability to pass into

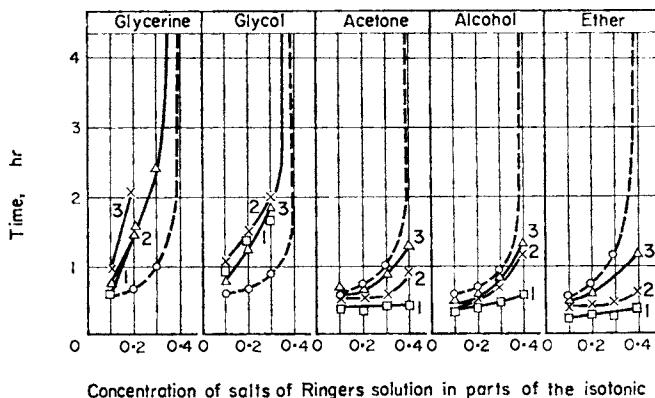


FIG. 125. The dependence of the life of muscles on the salts' concentration in Ringer's solution (R) in the presence of non-electrolytes and in their absence.

The continuous lines correspond to the solutions: glycerol—1—1·086 M + R, 2—0·543 M + R, 3—0·272 M + R; glycol—1—0·806 M + R, 2—0·403 M + R, 3—0·203 M + R; acetone—1—0·800 M + R, 2—0·418 M + R; ethyl alcohol—1—1·087 M + R, 2—0·543 M + R, 3—0·272 M + R; ether—1—saturated solution + R, 2—0·540 M + R, 3—0·270 M + R. The broken lines correspond to Ringer's solution (control).

cells, it was natural that the question should be raised whether in fact this phenomenon was not due to a direct effect of the non-electrolytes on the process of precipitation of the globulins or the similar muscle proteins—myosin. To clarify this issue experiments were performed in which a study was made of the effect of the same non-electrolytes on the precipitation of the globulins in serum when the salts concentration was diluted.

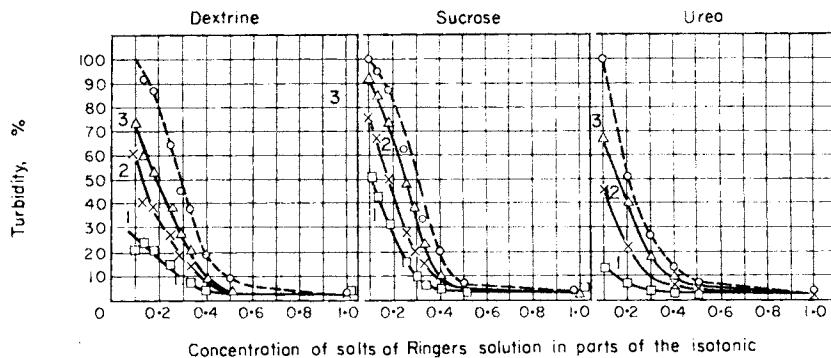


FIG. 126. The dependence of the turbidity of serum on the concentration of Ringer's salts with and without non-electrolytes.

The continuous lines correspond to the solutions: dextrose—1—0·035 M + R, 2—0·018 M + R, 3—0·011 M + R; sucrose—1—0·520 M + R, 2—0·275 M + R, 3—0·143 M + R; urea—1—1·667 M + R, 2—0·833 M + R, 3—0·417 M + R. The broken lines correspond to Ringer's solution (control).

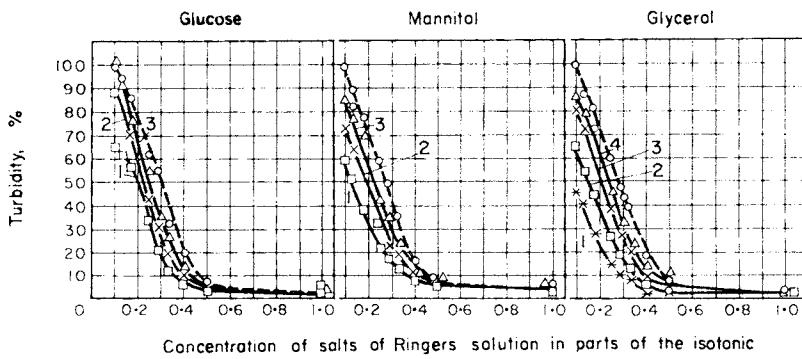


FIG. 127. The dependence of the turbidity of serum on the concentration of Ringer's salts with and without non-electrolytes.

The continuous lines correspond to the solutions: glucose—1—0·552 M + R, 2—0·275 M + R, 3—0·164 M + R; mannitol—1—0·750 M + R, 2—0·528 M + R, 3—0·272 M + R; glycerol—1—3·32 M + R, 2—1·883 M + R, 3—1·008 M + R, 4—0·523 M + R. The broken lines correspond to Ringer's solution (control).

Horse blood serum was diluted 10 times with a solution containing various concentrations of Ringer's salts with the addition of a quantity of a non-electrolyte. In the control experiments the serum was mixed with Ringer's solutions containing no non-electrolytes. An hour later the degree of coagulation of the serum globulins was determined with a Pulsfrich nephelometer (turbidimeter). The results of this series of experiments are shown in Figs. 126-9.

The control curves of the serum turbidity show that the globulins begin to fall out of solution at the same degrees of dilution of the Ringer's salts as that at which there is observed to be a sharp drop in the life of the muscles. The curves of the turbidity of the experimental samples with the non-electrolytes show that the substances which protect the muscles from the destructive effect of salt hypotony also protect the serum globulins from coagulating. The substances tested here give the same series in respect of their protective power as was obtained in the experiments with muscles (with the exception of urea), namely: dextrose > sucrose > urea > glucose > mannitol > glycerol > glycol.

Alcohol, ether and acetone in this case too only reinforce the effect of salt dilution.

Thus the data obtained, so it seems to me, enable us to interpret the protective action of the above named substances on salt hypotony as a direct stabilising effect on the cell proteins.

Krogh and Lindberg (1944) on the basis of their experiments with isolated frog hearts reached the same conclusion as that reached in my 1939 article, namely: the impermeability of the membrane for salts and sugars cannot be used as an explanation of the protection of cells from damage when immersed in a medium where a considerable quantity of the salts has been replaced with non-electrolytes. They found that if of the 124 mm of electrolytes in Ringer's solution 88 mm of sodium chloride is replaced with a like quantity (in mm) of glucose or sucrose, isolated frog heart works in such a solution exactly as it works in normal Ringer's solution. They found also that in Ringer's solution where part of the sodium chloride has been replaced by sugar, the carbohydrate passes into the muscle fibres and sodium ions are released from them. In the opinion of Krogh and Lindberg, the protective action of sugars in this case is due to the fact that the replacement of part of the sodium chloride in the medium leads to the displacement of a corresponding fraction of the ions of this salt in the muscle fibres by carbohydrate. However, these workers do not even attempt to explain how a non-electrolyte passing into a cell can replace the insufficient amount of salts in it.

It is known that the protective effect of non-electrolytes is seen not only when there are dilute saline solutions acting on the cells and the protein solutions, but also in the presence of other agents. Thus, Bryukhanenko and Bryukhanenko (1928) discovered that in the presence of a number of non-electrolytes protein does not coagulate even on heating to 100° and cannot

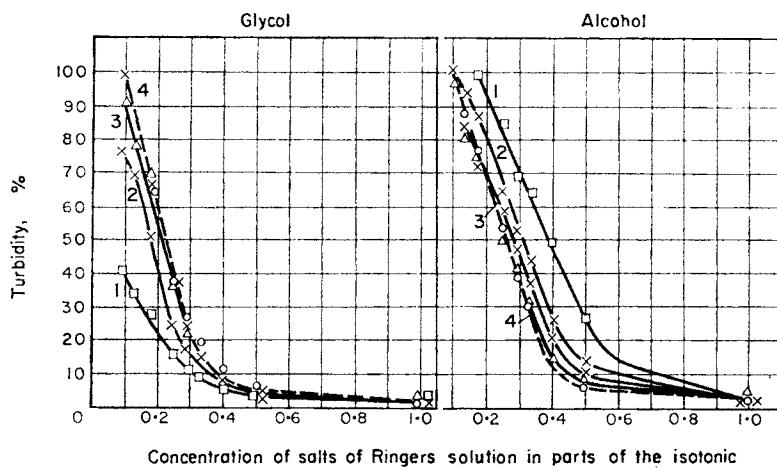


FIG. 128. The dependence of the turbidity of serum on the concentration of Ringer's salts with and without non-electrolytes.

The continuous lines correspond to the solutions: glycol—1—4·808 M + R, 2—2·764 M + R, 3—1·489 M + R, 4—0·776 M + R; ethyl alcohol—1—1·932 M + R, 2—1·004 M + R, 3—0·628 M + R, 4—0·209 M + R.

The broken lines correspond to Ringer's solution (control).

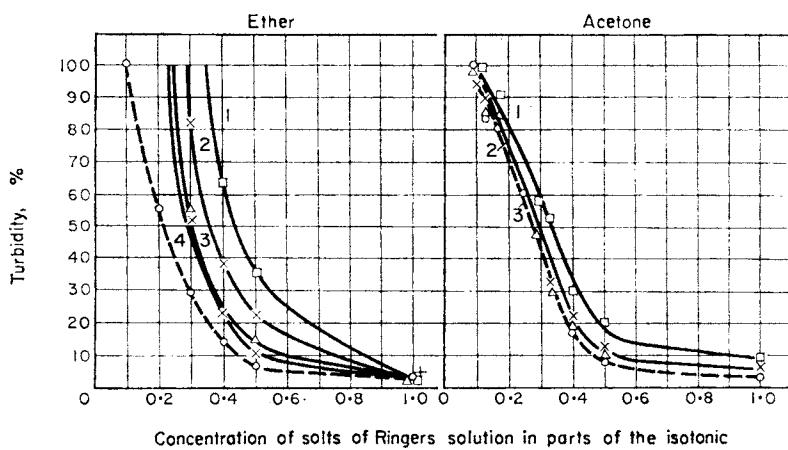


FIG. 129. The dependence of the turbidity of serum on the concentration of Ringer's salts with and without non-electrolytes.

The continuous lines correspond to the solutions: ether—1—saturated solution + R, 2—0·540 M + R, 3—0·270 M + R, 4—0·135 M + R; acetone—1—1·533 M + R, 2—0·796 M + R, 3—0·418 M + R. The broken lines correspond to Ringer's solution (control).

be precipitated with alcohol. The same protective effect is produced by glycol, glycerol, sugars and other substances containing OH-groups in their molecule. The authors note that the stabilising effect is greater the greater the molecular weight of the substance. Strong protective action by glycerol and sucrose was observed by Beilinsson (1929) upon heating solutions of egg albumin and rabbit serum. Kulikoff (1927), and also Kulikoff and Secretova (1927) discovered a similar effect of certain substances on heating diphtheria toxin and anti-diphtheria serum. Protein solution can be protected from denaturation not only by sugars (Putman, 1953), but also by ascorbic acid (Gorbacheva, Bresler and Frenkel', 1957), adrenaline (Sutulov, 1940b), ATP (Lyubimova and Engel'gardt, 1939) and other substances. Utkina (1938, 1939) showed that methyl, ethyl and amyl alcohol, acetone, glycol, glycerol, glucose and galactose in small concentrations depress the hypotonic haemolysis of rabbit erythrocytes, the strongest depressive effect being observed in solutions of the last two substances. According to her data, glycerol, glucose, galactose, maltose and salt hypertony protect erythrocytes from temperature haemolysis and lower the turbidity of horse serum on heating.

Fel'dman (1959) found that sucrose, lactose and galactose in nonplasmolysing concentrations enhance the temperature stability and resistance to high hydrostatic pressure of cells of the epidermis of *tradescantia* leaf. In plasmolysing concentrations of the same sugars the temperature stability drops and the resistance to hydrostatic pressure increases sharply; in order of their stabilising effect the sugars form the following series: lactose > sucrose > glucose > galactose. The sugars also increase the stability of these cells against the action of cold. A possible mechanism of the stabilisation of cells in these circumstances, connected with the denaturation of the proteins, is discussed in an article by Aleksandrov, Lyutova and Fel'dman (1959). Ushakov (1959b) attaches great importance to the action of the substances as cell protein stabilisers in increasing the cells' resistivity to the agents in the external medium.

Consequently, the phenomenon of the protective action of non-electrolytes presents a problem embracing a wide range of facts which can be explained from the point of view of the effect of these substances on the state of the protoplasm as a whole, and especially on the state of its protein component.

A large quantity of material has already been accumulated which points to the ability of many non-electrolytes to protect native proteins from denaturation and coagulation, but the causes of this phenomenon have not yet been clarified. I have myself (1939) made the suggestion that the mechanism of the stabilising effect of a non-electrolyte on the globulins upon dilution of the salts concentration is similar to the mechanism of the action of a protective colloid. It may be supposed that the non-electrolyte causing the stabilising effect is adsorbed in the micelle proteins and somehow protects them from coagulation and, at the same time, from destructive changes of the whole protoplasm.

## Conclusion

THE careful study of the water balance of live cells undertaken by various scientists has shown that animal and non-vacuolar plant cells do not have perfect osmometric properties. Osmometric properties are evidently attributable only to the vacuoles of plant cells which have a well established tonoplast.

Further, numerous workers have proved that all those substances which, according to the classical membrane theory, should not penetrate the protoplasm of resting plant cells and animal cells (carbohydrates, amino acids, polyhydric alcohols, salts of strong mineral acids and bases etc.) do in fact penetrate cells.

As a rule, at low concentrations the cell absorbs a greater percentage of the substance from the total quantity in the medium than it does at high concentrations. This phenomenon is due to the fact that the substance passing into the cell can be present in it in the bound (chemically or adsorptionally) and dissolved forms. In a number of experimental investigations it has successfully been established that in the state of diffusion equilibrium the dependence of the concentration of a substance in the cell ( $C_c$ ) on its concentration in the medium ( $C_s$ ) can be expressed by the formula:

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

where  $K$  is a constant showing how many times the solubility of the substance in one phase (in the cell) differs from its solubility in the other phase (in the medium),  $A_\infty$  and  $a$  constants characterising the limit and the steepness of rise of the isotherm of adsorption or chemical binding.

It follows from this formula that the greater  $C_s$ ,  $A_\infty$  and  $K$ , the greater the value of  $C_c$ , and the smaller the constant  $a$ , the smaller will be the concentration of the substance in the medium at which its binding by the cell will reach its limit.

In certain conditions in the case of some substances, as we have seen, it is possible to determine the numerical value of all the constants of this equation and, consequently, to calculate how much of the total amount of a substance penetrating a cell is in it in the dissolved and how much in the bound form.

In Table 104 we list those chemical compounds for which experimental conditions permit the determination of the values of  $K$  and  $A_\infty$ , on which,

as follows from the formula, depends the amount of the substance absorbed by the cell from the surrounding medium.

It can be seen from the table that the coefficient  $K$ , which characterises the difference between the solubility of substances in the cell and their solubility in the surrounding aqueous medium is considerably less than unity, varying within the limits 0·08–0·55.

TABLE 104. VALUES OF THE CONSTANTS  $K$  AND  $A_\infty$ .

Subject	Substances	$K$	$A_\infty$ (in mm per 100 ml of cell water)
Coacervates	Galactose	0·61	1·22
	Sucrose	0·60	0·64
Yeast cells	Lactose	0·55	6·43
	Chloride ions	0·45	0·00
Rabbit erythrocytes	Galactose	0·42	0·16
	Arabinose	0·43	0·60
Frog calf muscles	Galactose	0·32	0·11
	Sucrose	0·29	0·03
	Alanine	0·40	0·33
	Creatinine	0·38	0·07
	Phenol red	0·25	0·003
	Potassium ions	—	12·1
	Potassium ions <sup>(1)</sup>	0·45	12·0
	Sodium ions	0·30	0·93
	Sodium ions <sup>(2)</sup>	0·30	1·20
	Lithium ions	0·21	0·00
Frog sartorius muscle	Calcium ions <sup>(3)</sup>	0·10	0·25
	Chloride ions	0·20	0·00
	Chloride ions <sup>(2)</sup>	0·30	0·00
	Inorganic phosphorus <sup>(4)</sup>	0·08	0·07
	Potassium ions <sup>(1)</sup>	0·48	20·9
	Potassium ions <sup>(5)</sup>	0·50	4·7
	Neutral red <sup>(6)</sup>	0·25	0·038
Holothurian retractor			
Frog sciatic nerve			
Cuttlefish giant axon			

Notes: Calculated from the data of: <sup>(1)</sup> Steinbach, 1937, 1940a; <sup>(2)</sup> Fenn, Cobb and Marsh, 1934/5; <sup>(3)</sup> Dranitskaya, 1960; <sup>(4)</sup> Pisareva, 1961; <sup>(5)</sup> Fenn, Cobb, Hegnauer and Marsh, 1934/5; <sup>(6)</sup> Vereninov, Nikol'skii and Rozental', 1961 (calculated per 100 g of wet weight of fibre). The remaining data are taken from my own work.

The data set out in the table indicate that the coefficient  $K$  for one and the same substance but for different objects varies in magnitude. In the case of galactose, for example, for erythrocytes  $K = 0·42$  and for muscles  $K = 0·32$ .

Of special interest is the fact that the coefficient  $K$  of different substances for the same object is not the same, differing markedly from unity. In order of increasing magnitude of  $K$ , as obtained in experiments with muscles, the substances in the table fall into the following series:

Inorganic phosphorus < calcium ions < chloride ions < lithium ions < phenol red < sucrose < sodium ions < galactose < creatinine < alanine < arabinose < potassium ions.

The low and varied values of  $K$  for different substances are due, in my opinion, to the fact that all the water in the protoplasm is somehow organised ("bound"), so that the protoplasm behaves as a phase in relation to the surrounding aqueous solution of substances. This is supported by the following facts:

- (1) the immiscibility of the protoplasm with the aqueous medium;
- (2) the sharp variation of the distribution coefficient  $K$  from unity;
- (3) the difference in magnitude of the  $K$  coefficient for different substances;
- (4) the independence of  $K$  on the concentration of substances in the surrounding equilibrated liquid;
- (5) the lower dielectric constant of the protoplasm in comparison with a normal aqueous solution.

The varying solubility in the protoplasm of some substances by comparison with others can also be explained if it is recognised that the protoplasm and coacervates are special organic liquids in which the water itself is in solution or its molecules form part of organic complexes, as has been supposed by some workers. In any case the differing solubility of substances in the protoplasm and in the aqueous surrounding medium cannot be explained by the existence in the cell of an always constant amount of "bound water" ("non-solvent space"), because this amount varies for different substances (for example, for phenol red it is 75 per cent, but for arabinose 57 per cent). In recent years a number of scientists have put forward concepts similar to ours about the special state of water in the protoplasm. Suggestions have been made that the water molecules of the protoplasm are available to different degrees for the solution of different substances (Bozler, Calvin and Watson, 1958; Bozler and Lavine, 1958; Bozler, 1959; Carlin and Hechter, 1961; Polleri, Menozzi, Norman and Hechter, 1961 and others).

However, the lowered concentration of a substance in the cell by comparison with the concentration in the medium may be due not only to its lower solubility in the protoplasm, but also to other factors, especially to the rate of enzymatic reactions (for those substances which are subjected in the cell to enzymatic transformation). If the rate of the enzymatic reactions is greater than the rate of entry of the substance into the cell, then this substance may not be found at all in the latter. This was demonstrated by Kolotilova and Engel'gardt (1937) in a study of the distribution of glucose between medium and rabbit erythrocytes, whose blood cells have a very high glycolytic activity.

The lower content of dissolved ions in the cell is due, probably, not only to the lowered solvent activity of the protoplasm, but also to Donnan equilibrium. For those compounds which do not participate in enzymatic reac-

tions and are not electrolytes, their solubility in the protoplasm is evidently the single factor governing the excess of their concentrations in the medium over their concentration in the cell. Of course, this factor should also affect the distribution of electrolytes and those substances which are subjected to enzymatic transformation in the cell.

Another factor which has a decisive influence on the extent of the absorption by a cell of many chemical compounds is the adsorptional and chemical activity of the cell colloids. The adsorptional and chemical binding by the protoplasm of different substances varies within very wide limits. Thus, the greatest amount which can be bound by frog muscles ( $A_{\infty}$ ) is 12·0 for potassium, 0·33 for arabinose, 0·11 for galactose and 0·03 mm for sucrose for an amount of dry residue of muscle corresponding to 100 g of intracellular water, while chloride ions are not adsorbed at all by the muscle colloids. Yeast cells adsorb very large amounts of lactose ( $A_{\infty} = 6\cdot43$  mm) and rabbit erythrocytes of galactose ( $A_{\infty} = 0\cdot16$  mm). Frog muscles absorb urea, neutral red and rhodamine particularly strongly.

It follows from Table 104 that in order of their increasing ability to be bound by muscle the substances investigated can be placed in the following series:

lithium and chloride ions <<< phenol red < sucrose < creatinine, inorganic phosphorus < galactose < calcium ions < alanine < arabinose < sodium ions < potassium ions.

If the solubility of these substances in the protoplasm differs by a factor of about 6, the magnitude of the limiting binding ( $A_{\infty}$ ) varies by a factor of 200 (from 0·003 mm for phenol red to 0·6 mm for arabinose). This difference is infinitely large in the inorganic ion series. It may be concluded from these data that the difference in the absorption of different substances by cells from the medium is basically due to their differing capacity to be bound by the protoplasm colloids.

If the first factor—solubility—(at least, for the chemical compounds investigated) only limits the process of absorption of these substances by cells, then the second factor—adsorptional and chemical binding—acts in the opposite direction, this action being the stronger the less the concentration in the medium of the chemical compounds being absorbed by the live matter.

Thus we may suppose that there are created in the cell the conditions for regulating the entry of substances. This supposition is natural because it has been proved that for many compounds which are able to be adsorbed to some extent by the protoplasm it is possible to find a small concentration at which their concentration in the cell will be greater than in the medium. At sufficiently large concentrations, when adsorptional binding can no longer cover the deficit in the cell caused by the lowered solvent power of live matter, the reverse ratio occurs: the concentration of these compounds in the cell is found to be less than in the medium.

I attach great biological significance to these physico-chemical factors and consider that it is they that ensure the uniform and adequate supply of nutritive substances to the cell within very wide limits of their content in the surrounding medium and thus create favourable conditions for the course of enzymatic reactions.

The material reviewed in the preceding chapters on the mechanism of the distribution of inorganic substances indicates that one part of these substances can be present in the cell in the firmly bound state (in the unionised form), while another part is less firmly bound with the cell colloids, apparently by adsorptional forces, this part being present in the form of ions, and a third part is in the dissolved state in the highly mobile cation and anion fraction. Probably not less than 50 per cent of the total "bound" potassium is present in ionic form in the protoplasm.

The low solubility of ions in the protoplasm and the high density of negative stationary (fixed) charges on the protein groups and other organic compounds bound with the proteins create conditions which ensure a low concentration of mobile anions in the protoplasm, such as chloride and other ions. The selective adsorption of potassium ions and its low hydration energy ensure a low sodium ion concentration there.

From the point of view of the sorptional theory developed here functional changes in cell permeability are caused by reversible changes in the sorptional properties of live matter, changes in the adsorptional and chemical activity of the protoplasm colloids and also in its activity as a solvent.

There are many facts which support the connection of the functional changes in cell permeability with changes in the properties of the principal component of live matter—the cell proteins.

It is known that of all the organic substances which go to make up the protoplasm the proteins are found in the greatest amounts. They do, in fact, play a most important role in the triggering off of all the functions of live matter. They owe this property of theirs to the fact that they have a colossal capacity for interacting with other substances of the most varied chemical nature (Gulevich, 1934; Pasynskii, 1948; Braun, 1949) and to the fact that they can easily alter these properties of theirs in relation to changes in the ambient conditions (Nasonov and Aleksandrov, 1940; Nasonov, 1959a; Ungar, 1957, 1959, 1961a; Braun, 1960; Ling, 1952, 1960).

It is customary to-day to connect a change in the capacity of the native proteins to interact with other substances with a change in the configuration of the protein molecule which takes place without destroying its integrity (without breaking the peptide bonds). It is considered that this leads to the appearance in the protein molecule of new active chemical groups. All these phenomena are described as denaturation changes of the proteins (Nasonov and Aleksandrov, 1940; Aleksandrov, 1947, 1948a, b, c; Talmud, 1948; Braun, 1949; Nasonov, 1949a, 1959a; Pasynskii, 1952; Pasynskii, Volkova

and Blokhina, 1955; Pavlovskaya, Volkova and Pasynskii, 1955; Ungar, 1959, 1961a; Belitser, 1960 and others)\*.

The great volume of information available indicates that denaturation changes of proteins in cells are reversible, the requisite energy being obtained by the cell at the expense of metabolism. It is at the expense of the latter too that the continuous renewal of the proteins of live matter takes place. The process of protein denaturation is connected with an increase in affinity for one substance and a decrease for another. Thus, it is known that upon denaturation the hydrophilic nature of proteins declines, their ability to bind some inorganic ions also falls though it is increased for others, while the binding of basic and acid vital dyes is always increased. Alterations of this kind in the protoplasmic proteins, so it seems to me, lie at the basis of the functional changes of cell permeability.

When excitants act on a cell, there is in the first place, as may be assumed, a reversible change in the chemical and adsorptional activity of the cell proteins, which leads to the breaking down of the complex structure of the protoplasm and increased solubility of substances in it. These changes are accompanied by reversible loss of affinity of the cell colloids for some substances and increased affinity for others, resulting in increased permeability for some substances and reduced permeability for others.

On excitation and reversible damage there also occurs a redistribution of mineral substances between cell and medium: potassium and phosphate ions are released from the cell, and sodium and chloride ions pass into it. When the live matter returns to normal, the potassium ions again pass into the cell, and the sodium and chloride ions are released into the medium. This phenomenon, as may be supposed, is related to reversible loss of affinity of the proteins for potassium and to the decomposition of the organic phosphorus compounds, which enables them to escape from the cell. In view of the fact that this is accompanied by reversible increase in the solubility of ions in the protoplasm, and, furthermore, sodium ions acquire an adsorptional affinity for the altered proteins, sodium and chloride ions pass into the cell, and, when the protoplasm passes out of the excited state or the damage is liquidated, their solubility in the live matter again falls, the affinity of the proteins for sodium ions is reduced and so their concentration in the cell drops. This process is connected with the expenditure of energy which the cell takes from metabolism.

On breakdown of metabolism (oxygen starvation, action of various meta-

\* On the structure of protein molecules and the essence of denaturation phenomena see the review articles of Pasynskii and Belitser (1953, 1954) and Bresler and Talmud (1954).

Belitser and Tsyporovich (1948, see also 1952) and Belitser (1949) consider that it is possible and necessary to separate the process of denaturation into two stages depending on the nature and depth of the changes in the protein molecule. Weak (superficial) and completely reversible changes, including apparently paraneurotic changes of live matter, are ascribed by them to the "predenaturation" stage. Pasynskii and Belitser (1953) go so far as to refuse the name of denaturation changes to such changes in protein molecules.

bolic inhibitors etc.) there also occurs a redistribution of inorganic substances between cell and medium (release of potassium from the cells and uptake of sodium). This phenomenon is also reversible when the cells are returned to normal conditions.

Apart from the noted redistribution of inorganic ions, excitation or damage gives rise to increased absorption of acid and basic vital dyes and transition to the free state of the nucleic acids, lipids and so on. At the same time, as was shown by Oparin, Kursanov and Sisakyan, many enzymes pass from the bound state to the free, in which connection the direction of the enzymatic process changes and biochemical reactions can take place which never occur in the resting protoplasm. The described mechanism of the redistribution of inorganic substances probably lies at the foundations of the cause of injury potentials (resting potentials) and action potentials.

Consequently, the materials discussed make it possible for us to describe the observed functional changes in cell permeability by shifts in the sorptional activity of the whole protoplasm and, in the first place, by changes in the sorptional properties of the cell proteins, with which the shifts in the biochemical and physiological activity of the protoplasm are closely connected.

There is a great volume of evidence in the literature that shows the sorptional activity of the different cell structures to vary, so that individual substances are absorbed by the protoplasm structures with non-uniform intensity. When external irritants and nerve impulses act on a cell, the sorptional properties of the various cell structures vary to differing extents, so that, in all probability, there is a redistribution of substances not only between cell and medium, but also within a cell between the cell formations (cytoplasm and nucleus, mitochondria and cytoplasm, cell shell and protoplasm, protoplasm and the contents of plant cell vacuoles and so on). This has been shown particularly convincingly in experiments with dyes by numerous authors.

So, from the point of view of the theory developed here, cell permeability depends on the sorptional activity of live matter as a whole. The published data and the results of my experiments show that the greater or lesser permeability of a cell is determined by the coefficient  $Q$  — the ratio of the concentration of a substance in the cell to its concentration in the medium in conditions of diffusion equilibrium. If a change in the functional state of a cell results in a reduction of this coefficient, a redistribution of the substance between cell and medium can occur with release of the substance from the cell to the outside.

The magnitude of the coefficient  $Q$ , as we have seen, depends on the solubility of the penetrating substance in the protoplasm and on the adsorptional and chemical binding of it by the cell colloids. The more strongly marked the adsorptional and chemical binding of the substance by the protoplasm and the smaller the concentration of it in the medium, the greater will be the coefficient  $Q$  and the greater will be the cell permeability constant.

From the point of view of the sorptional theory, the division of cell

permeability into "physical" and "physiological", which was proposed by Höber, or into passive permeability and active transport governed by carriers and enzymatic systems in the cell membrane, is, in our opinion, as erroneous as the reduction of permeability to a diffusion process. "Physiological permeability" covers only those cases when the movement of a substance into or out of a cell is observed to take place "against the concentration gradient". Only this process can be said to be effected by the cell "actively", with the expenditure of a definite amount of energy. All the other cases, when the diffusion of a substance takes place without the expenditure of metabolic energy ("along the concentration gradient") are covered by "physical" permeability.

In reality, the movement of substances into and out of cells is always effected "along the concentration gradient". The diffusion of a substance in the system cell/medium always takes place in the direction where the concentration of its dissolved fraction is less than that which should be established at diffusion equilibrium. The diffusion of a substance into a cell "against the concentration gradient" occurs because part of it is bound there adsorptionally or chemically, while the movement of substances out of a cell into the medium takes place because they are liberated in the cell from the bound state and because their solubility in the protoplasm is less than in the medium. Metabolic energy is here needed in order that the sorptional properties of live matter (adsorptional and chemical activity and dissolving power of the protoplasm) are maintained on a definite level.

However, the cells of the secreting and resorbing epithelia in fact perform the function of active transport. They transfer certain substances from one medium to another frequently against the concentration gradient, but this function of theirs is achieved not by any cell membrane mechanisms (or not only by them), but by the cell as a whole; the cell organelles (mitochondria, Golgi's apparatus etc.) indubitably participate in this process.

A critical review of the membrane theory shows that it is contradictory to many facts. Therefore we must consider incompetent its claim to universality, to explain from a single (the membrane) point of view a number of vital biological phenomena—the regulation of metabolism, excitation and the transmission of nerve impulses, bioelectric potentials, narcosis and so on. All these phenomena are connected by the membrane theory with a membrane to which are ascribed the most varied and occasionally unreal properties.

The sorptional theory starts from the basis that the permeability of cells, as being a most important aspect of metabolism, like all the other biological phenomena mentioned above, is to be explained by the properties of the protoplasm itself.

In conclusion, it should be noted that in the general problem of cell permeability there remain many important questions which are yet unanswered, among them some of very great difficulty. It is my deep conviction that the sorptional theory of cell permeability will play a positive role in the solution of some of these questions.

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