THE PERMEABILITY OF NATURAL MEMBRANES

By HUGH DAVSON and
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With a Foreword by
E. NEWTON HARVEY

Cambridge University Press

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FOREWORD

By E. NEWTON HARVEY, *Professor of Physiology, Princeton University, U.S.A.*

JUST as chemistry could not have developed without test tubes to hold reacting substances, so organisms could not have evolved without relatively impermeable membranes to surround the cell constituents. This barrier between the inside and the outside, the inner and external world of each living unit, has been and always must be considered one of the fundamental structures of a cell. No one can fail to be impressed with the great difference in properties of living and dead cells. The dead are completely permeable to diffusible substances, while the living retain one material and pass another. This difference, selective permeability, is so marked that it becomes the surest test to distinguish the living from the dead, holding where all other methods fail. It can truly be said of living cells, that by their membranes ye shall know them.

There can be no doubt of the fundamental importance of cell permeability. Several symposia on membranes have been held recently, notably that of the Faraday Society in 1937 and that at Cold Spring Harbor in 1940, but no general books on cell permeability have appeared for over ten years. It was, therefore, with great pleasure that I learned of Drs Danielli and Davson's plan to bring together the existing knowledge in a book dealing with natural membranes. Such an undertaking is no mean task. Cell permeability has passed from the qualitative to the quantitative stage and the detailed data now available would baulk less enthusiastic authors, even in normal times. The compilation will be even more appreciated in that it has been carried out under the stress of war. Cell physiology will be grateful indeed for this summing up of a subject which is destined for rapid development under the stimulus of modern methods of exploring molecular dimensions and molecular arrangement. Viewpoints may differ but the facts remain. These are systematically and logically presented in this timely volume.

PREFACE TO SECOND EDITION

THIS book was first published when the great majority of British biologists were actively engaged in war-time research; somewhat surprisingly—to the authors at any rate—the edition sold out within a year; and since then the frequent enquiries as to when a new edition would be available have prompted the reprinting of this book with a few minor additions. A completely revised edition, incorporating all the most recent work, would, of course, have been more satisfactory; but we have refrained from undertaking this, partly because of other literary commitments, but principally because the time is not yet ripe. The introduction of radioactive isotopes into the study of the active transport of ions across cellular membranes marks a great step forward in the investigation of this aspect of permeability; because of the war and its aftermath, however, it is only within the last year or two that research with the aid of this tool has really got into its stride; and it may be confidently predicted that the next five years will show striking advances in our knowledge of ionic permeability and its relationships with metabolism. Until then, a full discussion of the subject would be premature; and this re-issue must be regarded as a stop-gap, providing the reader with a summary of facts and interpretations that must now be described as classical, and containing, in the form of brief addenda at the end of each chapter, a few pointers to the more significant results published within the past ten years.

H.D.
J.F.D.

AUGUST 1951

AUTHORS' PREFACE

In this book we endeavour to give a general survey of the field of permeability. We have included materials essential for students of Medicine, Physiology, Biochemistry, Zoology and Botany, and have provided key references so that the literature on any point which it is desired to pursue further may be looked up with a minimum of trouble. We hope this will assist those lecturing on permeability, and will accelerate the disappearance of the many errors which have crept into the literature designed for students.

The last twenty years have seen a steady development of exact measurements of membrane permeability, mainly due to the American schools of Lillie, Lucké and McCutcheon, and Jacobs, but also largely contributed to by the Finnish school of Collander and Bärlund. To-day we may on the one hand say that the experimental side of this field is now mainly quantitative. During the same period physical science has made many advances in the fields of surface chemistry and the structure of liquids and solids. These have provided us with the basic materials for a quantitative theory of permeability. To some extent we have incorporated such a theory in this book, drawing principally on the work of I. Langmuir, W.D. Harkins, N.K. Adam, E.K. Rideal and E.N. Harvey when dealing with membrane structure, and on the theory of activated diffusion and on such work as that of Fowler & Bernal in achieving a description of the process of penetration of a membrane. So that, on the other hand, the theory of permeability has begun to take a quantitative form. We believe that we may now definitely claim that permeability studies have passed beyond the preliminary exploratory stage, and have reached the stage at which quantitative analysis is of dominant importance. This first attempt at such analysis will, we hope, merely be the precursor of a more exact study.

As J. Loeb complained many years ago, obscure or inexplicable phenomena in biology are fashionably brought into the currency of "knowledge" by way of the philosophers' stone "a change in permeability". When to this the more modern elixir of "surface action" is added, night unto night sheweth knowledge. Such speculations serve a useful purpose in giving an apparent

coherence to scattered and isolated observations, so encouraging the collection of more facts. But the sooner superficialities are replaced by a detailed understanding of underlying mechanisms, the better for science. We hope that this book will assist in defining what can, and what cannot, be done by the cell membrane, by "surface action" and by "changes of permeability". We have not included a chapter on monolayers, etc., since the books of Adam and Rideal are a much more adequate introduction to the necessary fundamentals of surface chemistry than we could hope to provide.

We wish particularly to thank the many friends who have helped us, both by friendly criticism and by providing us with laboratory facilities to carry out our studies—Professor J. C. Drummond, Professor A. V. Hill, Professor C. Lovatt Evans, Professor Sir Frederick Gowland Hopkins, and Dr J. Needham in England; and Professor E. N. Harvey, Professor M. H. Jacobs and Dr E. Ponder in America. We have also, at various times, been assisted by the advice and criticism of Professor J. D. Bernal, Dr A. C. Burton, Professor R. Collander, Dr L. H. N. Cooper, Dr S. L. Cowan, Dr H. J. Curtis, Dr C. Goodeve, Professor R. Höber, Professor D. Keilin, Dr M. Maizels, Dr D. Mazia, Professor W. J. V. Osterhout, Dr A. K. Parpart, Dr J. H. Quastel, Dr F. J. W. Roughton, Dr H. Shapiro, Dr J. H. Schulman, Mr F. J. Turton, Dr W. Wilbrandt. No one mentioned here, however, do we wish to burden with responsibility for any views advanced in this book, except where expressly stated.

We are also indebted to Mr J. F. Danielli, Senr., who read the proofs, to Dr A. Neuberger, who read part of the proofs, to Dr W. A. H. Rushton, who read the proofs of Chapter xv, and to Mrs Mary Danielli for assistance with the manuscript, proof and index.

The tardiness of communications, due to the war, has prevented full discussion of some points, so that responsibility for the views advanced in the individual chapters must be mainly borne by one or the other of us.

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AUGUST 1940

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Note. Readers primarily interested in conclusions can profitably omit reading Chapters II, III and IV

NOTE

In places it has been necessary to include unpublished results or calculations. Those by H.D. are marked **, those by J.F.D. ††

CHAPTER I

SIGNIFICANCE OF PERMEABILITY STUDIES

THE distinction between the interior of a cell and the medium which surrounds it is maintained by a membrane about one millionth of a centimetre in thickness. Continued existence of the cell is dependent on the ability of this membrane to permit passage of some substances and prevent that of others. For the more complex organisms other membranes become essential, such as those of the blood capillary, the glomerulus of the kidney, the blood-brain barrier, etc. The study of these various membranes is a basic branch of physiology. From its study information of two kinds is obtainable: (1) knowledge of the structure of the membranes; (2) knowledge of part of the mechanism whereby living bodies maintain their composition and ability to function. By measuring the permeability of cells to various substances we obtain information which enables us to predict the relative rates at which various types of molecules will penetrate into cells. Then, confronted with, say, a group of drugs, we should be able to predict that some will penetrate rapidly into cells and others very slowly, if at all. Thus we are able to define an important group of drugs whose action must be on the surface of cells, since they do not penetrate sufficiently rapidly to produce their action on the interior of cells. A similar problem arises with metabolites. It will be our task here to analyse experimental observations of permeability so that a straightforward answer may be given to such practical questions. In doing so we find that substances fall roughly into two main groups: (*a*) substances which diffuse according to the laws of thermodynamics, only from a region of higher to one of lower concentration,* so that in the final equilibrium condition the substance is in the same concentration on both sides of the membrane; (*b*) cases where the laws of thermodynamics are apparently broken and molecules accumulate on one side of a membrane, in excess of the amount on the other side.

* To be exact we should write *chemical potential*, not *concentration*. According to the Second Law of Thermodynamics a substance diffuses from a region of higher to a region of lower chemical potential. In biological systems concentration is usually proportional to chemical potential, and thus may be used instead.

As an example of this latter phenomenon may be taken the frog kidney tubule: the fluid entering the tubule from the glomerulus has approximately the same concentration of chloride ion as the blood plasma, but practically all of this chloride ion diffuses across the tubule membrane into the vascular system, against a concentration gradient, so that the urine is practically chloride free. As it is highly improbable that the Second Law of Thermodynamics is actually broken, we at once infer that in such cases the cells concerned supply energy for the transport of molecules. Thus the two groups of molecules are really (*a*) those towards which the behaviour of the cell membrane is passive, and (*b*) those towards which it is active. The former group of substances is much the larger, and the details of the mechanism whereby these substances pass through the cell membrane are now fairly well known. The second, smaller, group of molecules, subject to secretory activity, accumulates in cells by mechanisms which are still almost completely unknown. It is very probable that the power of a cell to accumulate or excrete certain substances and not others, against a concentration gradient, is intimately connected with the structure of the membrane. The accumulation or excretion must, of course, also be connected with the special nature of the cell's metabolic processes, and it is of fundamental importance to determine the nature of this metabolism. The problem of secretion, therefore, presents a problem to the specialist in cell permeability as well as to the specialist in metabolism; the study of the nature of the membrane, running hand in hand with the study of the special nature of the metabolic processes involved in secretory activity, will eventually lead to the solution of many of the problems of secretion and growth.

The connection between changes in cell membrane permeability and the function of the cell is not clearly defined; however, some interesting correlations have been obtained. Definite changes in the rate of penetration of alkalies into marine eggs on fertilisation were established as long ago as 1911 by E. N. Harvey, and since then changes in permeability to water and certain dissolved solutes have also been established by various workers. Cyclical changes in the permeability of the developing egg, correlated with successive divisions, have been claimed to exist by Herlant (1918). O. Warburg, in a series of investigations on the oxygen consumption of cells, has shown that changes in the membrane are

often correlated with large changes in the oxygen consumption (*vide* Warburg, 1910). Again, it has been shown that narcotic substances, i.e. substances which depress the metabolic activity of cells, will, in the same concentrations in which they exhibit this narcotic effect, also depress the permeability of certain cells to penetrating substances. But narcotic substances may also increase cell permeability and it has yet to be shown that there is, in fact, any direct functional relationship between permeability and narcotic action. Then again, it is believed that the contraction of a muscle fibre is a response to a transient increase in permeability of the fibre plasma membrane. But in most of these examples, as in the others which could be given, experimental evidence is slender and the theoretical basis tenuous. Consequently, in the following chapters, we shall deal mainly with experimental results and with membrane structure, while relationships with other physiological fields will be only roughly indicated.

Now let us consider the precise meaning of the phrase "permeability of a membrane". We can express this quantitatively as the amount of substance in gram mols penetrating in a given time. Obviously the area of the membrane will be important and also the concentration difference across the membrane. The unit of area most convenient for biological systems is the square micron, so that by "the permeability of a membrane to a substance" we refer to the net number of gram mols of that substance diffusing through an area of one square micron of membrane in one second, per gram mol per litre concentration difference across the membrane. No reference is made to the thickness of the membrane in defining its permeability, but on the other hand the temperature has to be defined. Thus the prime variables which must be defined in experimental work are (1) time, (2) membrane area, (3) concentration difference across the membrane, (4) temperature, and (5) pressure.

The area of the membrane available for diffusion is a quantity which in all permeability studies is equated to the geometrical area of the cell membrane, determined by microscopic examination: this is done simply from lack of information regarding the detailed structure of most, if not all, cell membranes. If the diffusion of the substance being studied is possible through all parts of the membrane, and if there are no submicroscopic convolutions of the surface, then this procedure is justified. Suppose,

on the other hand, that the membrane has a single pore, say one hundred times the diameter of the penetrating molecule in size, but nevertheless occupying, say, one ten-thousandth of the total area of the membrane, and that the remainder of the membrane is completely impermeable. Experimentally it would be found that diffusion through the membrane would occur at about one ten-thousandth of the rate across a similar cross-section of the surrounding medium, i.e. the differences in permeability of the membrane to different substances are in this case due to variations in the rate of diffusion through the water in the pore, not to variations in the interaction of the molecules of the membrane and of the penetrating substance. The various mathematical tests which may be applied to permeability data to determine to what extent retardation is caused by the limited proportion of the total area of the membrane available for diffusion, as opposed to the factor of molecular interaction, will be discussed in later chapters, and it is sufficient to point out here the possibility of obtaining illusory results regarding the latter factor by ignorance of the former.

CHAPTER II

METHODS OF STUDYING MEMBRANE PERMEABILITY

By J. F. DANIELLI and H. DAVSON

THE subjects used for permeability studies may be divided into two groups. In the first group are cases where the penetration of substances into the cytoplasm is observed, so that the barriers to diffusion are the enveloping membranes of the cell, of which in most cases the plasma membrane only is of importance. In the second group fall relatively inanimate membranes, such as the chitin membrane of crustacea, and more complex membranes, such as frog skin and the capillary membrane, in penetrating which a molecule has a choice of several paths, either passing between cells or passing through the interior of the several sorts of cell composing the membrane: in the latter event a penetrating molecule must pass at least twice through a cell plasma membrane before it has passed through the complex membrane.

GROUP I

Into this group fall erythrocytes, marine and other eggs, leucocytes, yeast, bacteria, plant cells, muscle cells, nerve axons, etc. Of these, plant cells, erythrocytes and marine eggs have been the most studied.

The Advantages of the Erythrocyte as an Experimental Object. The erythrocyte is used as a subject of permeability studies for a variety of reasons, the chief of which are:

(*a*) Its availability.

(*b*) It contains a high concentration of a pigment which escapes when the cell membrane is stretched beyond a certain point, so that any swelling beyond this point can be immediately and quantitatively detected.

(*c*) A small volume of blood, say 1 ml., contains about 5×10^9 cells, apparently with their size and other properties varying continuously and normally, so that the study of such a large

number in any given sample will represent the mean behaviour of the erythrocytes of a given animal with a considerable degree of accuracy.

(*d*) It is a more robust cell than are e.g. marine eggs, and it may be centrifuged at high speeds without serious damage (there are exceptions to this).

(*e*) For moderate volume changes it increases in volume without increasing in area, owing to its special shape, so that the equations describing the rates of penetration of a solute or water into the cell are much simplified.

For these reasons a great deal of work has been done on this cell, and furthermore that which has been done is generally of a more quantitative nature than many studies on other cells.

Methods of Measurement of Permeability. The various methods used may be separated into three groups: (1) those cases in which the amount of penetration is estimated by a direct chemical procedure; (2) cases in which volume changes are studied by some physical measurement; (3) estimation of rates of penetration by spectroscopic methods. Of these groups, chemical methods only are wholly reliable, though usually tedious. Physical methods, when used with discrimination, have many advantages technically, but are more apt to give misleading results.

The Chemical Method. This is a direct method, which, provided the chemical estimation is specific, is the one to be chosen whenever practicable. The substance to be studied may be added directly to the whole blood, and after definite times samples of the blood may be removed, centrifuged, and either the cells or supernatant fluid analysed. Alternatively, samples of the cells may be added directly to a solution of the penetrating substance in Ringer or serum and later centrifuged down and the cells or fluid analysed.

Remarks. Suppose the substance is added to whole blood, and the serum is to be analysed. The presence of the penetrating substance causes a continuous change in the volume of the cells, and therefore a change in the concentration of the substance in the plasma *due to water shifts*, apart from changes caused by penetration into the cells. These may be accounted for by haematocrite determinations (see p. 7), but this introduces a fairly large error. This difficulty may be overcome by estimating

the concentration inside the cells instead of that in the serum, provided all the cells in a given sample of the original blood are taken (Davson, 1934). Here, however, another objection is introduced; since it is impossible to exclude the plasma completely from the cells, a considerable error is introduced by the inclusion of quantities of serum, which contains a high concentration of the penetrating substance, with the cells. With slowly penetrating substances, however, this difficulty may be overcome by washing the cells with a Ringer's solution after the given times have elapsed. Davson has applied the method successfully to the penetration of potassium into the cat cell; in this case, if cells are suspended in isotonic KCl, potassium penetrates the cells to cause a rise in the concentration of potassium from 30 to about 100 mgm. per 100 ml. H_2O in 1 hour. The interstitial KCl may then be removed by washing with ice-cold NaCl.

Other objections are the inaccuracy in the measurement of the times of the actual suspension of the cells, owing to the uncertainty in the evaluation of the actual time of suspension during centrifuging (this proves not to be very important) and also the necessity for centrifuging which may produce changes of itself (Davson & Danielli, 1938). The method is, of course, not applicable to rapidly penetrating substances (see, however, Dirken & Mook, 1931).

Cryoscopic measurements of the changes in concentration of the serum may be made in place of the chemical determinations (Hedin, 1897); this, however, considerably reduces the accuracy of the method.

The Method of Measuring Volume Changes. If cells are placed in an isotonic Ringer's solution to which the penetrating substance is added, they will first shrink, due to loss of water, and subsequently swell, due to gain of water, as the substance enters the cells. The rate at which the swelling occurs will give a quantitative estimate of the rate of penetration of the substance considered. The measurement of the volume changes may be made by use of the haematocrite (e.g. Mond & Hoffmann, 1928), i.e. centrifuging a sample in a graduated tube and measuring the height of the column of cells, or by diffraction methods if the cells are first converted to spheres (Ponder, 1934), or by the dispersion of light measured with a photo-electric cell (Örskov, 1933). The haematocrite is not a reliable instrument (Ponder, 1934); but the dispersion

of light seems to be a good index to the relative volume changes of the cells.

Haemolysis Method. It has long been known that if a cell is placed in a solution of a penetrating non-electrolyte, it swells until it bursts, i.e. haemolyses; the rate of haemolysis may be used as an index to the rate of penetration. This method was first used by Griyns (1896) and is now widely used for non-electrolyte studies. The degree of haemolysis is usually measured by an optical method based on the change in the degree of scattering of light by a suspension of cells, after the bursting of the latter. Thus if we mix one drop of blood with 25 ml. of an isotonic solution of a penetrating non-electrolyte, e.g. 0·33 *M* glycerol, in a boiling tube, it is found that on holding the tube in front of a luminous filament, or other bright body, the latter cannot at first be seen: as haemolysis proceeds the suspension becomes less opaque, until at the stage when about 75% haemolysis has occurred the filament becomes distinctly visible.

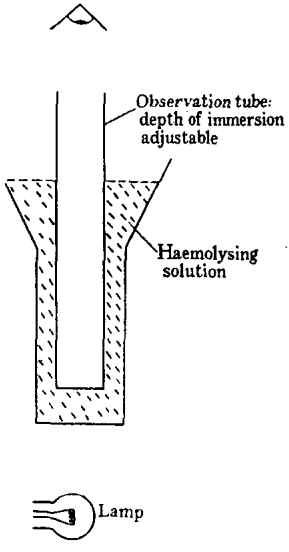

FIG. 1. Diagram of apparatus used by Jacobs for determining percentage of haemolysis.

This principle has been utilised by Jacobs (1930), who describes a method in which the minimum depth of a suspension of erythrocytes necessary to prevent perception of a glowing filament is observed. The filament is at standard brightness. As haemolysis proceeds, the light-transmitting powers of the suspension improve, and the minimum depth increases. The time course of the change in depth is recorded on a kymograph. Alternatively, the instrument may be set so that when the filament is just visible a known degree of haemolysis has occurred; the time required for this to occur is used as an index to the rate of penetration of the non-electrolyte (Fig. 1).

We have already seen that a given erythrocyte can swell up to a certain size, and will then haemolyse. But not all erythrocytes haemolyse at the same tonicity. Fig. 2 shows the percentage of

haemolysis of ox erythrocytes after 1 hour in NaCl of various concentrations. Jacobs (1932) has worked out the relationships between the permeability to water, the osmotic concentrations inside and outside the cell, and the time required to reach a given degree of haemolysis (see Chapter IV). From this the permeability constant to water may be calculated by observing the time required to reach, say, 75% haemolysis in either water or hypotonic solutions of non-penetrating substances, such as NaCl or sucrose.

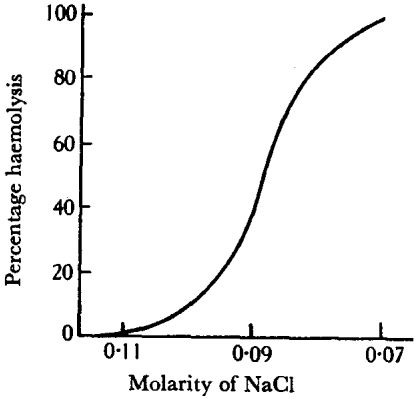

FIG. 2. Percentage haemolysis of ox erythrocytes, plotted against tonicity (after Jacobs & Parpart, 1931).

Where substances other than water are concerned the situation is more complicated, since the penetrating solute and water are penetrating simultaneously. Jacobs (1934) has discussed this in some detail, and has evolved a system whereby, if the value of the permeability, P_w, to water is known, it is sufficient to determine t_s/t_w, the ratio of the time taken in reaching a given percentage of haemolysis in isotonic solute to that taken in water. Then the corresponding value of P_s/P_w is read off from a graph, and, since P_w is known, P_s, the permeability to the solute, can be calculated.

However, most workers seem to have found it sufficient to use the times for the cells to reach a given degree of haemolysis, say 75%, as a measure of the relative rates of penetration of different substances. This is only accurate for times of haemolysis in excess of 1 min.