RECENT ADVANCES IN PHYSIOLOGY

Eighth Edition

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With a Foreword by

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FOREWORD

I HAVE been asked to write a Foreword to this book as I wrote the First Edition of it thirty-eight years ago, and produced three further Editions during the succeeding five years. In preparing the book, I made it my aim to select subjects which could be regarded as growing points, from which further developments could be expected, and which might enable advanced students more easily to keep abreast of these. With the exception of one chapter I wrote the book single-handed. That exception was the chapter on the Conditioned Reflexes which was done for me by Dr. Anrep and was the first account of the subject to appear in the English language.

The next Editor was my friend the late Professor W. H. Newton, who carried on along the same lines, and also single-handed, to the Seventh Edition. He was suffering from his fatal illness at that time, and the Edition was not fully completed. That was in 1949, and in the intervening years no Editor has come forward to continue the publication.

In the meantime we have been overcome by the flood of literature to the point of submersion, so that it is now impossible for any single person to undertake it. Hence the book has reached the Committee stage, and has to be produced by a team. This is, I think, no loss for a book of this description, because each contributor is an expert in the field on which he writes. And the general Editor, Dr. Richard Creese, is to be congratulated on the team selected and the contributors on the treatment they have used in expounding their subjects. I feel sure that the volume will form a useful bridge between text-book and detailed monograph, and will be much appreciated.

C. LOVATT EVANS

Winterslow,
Wilts.

PREFACE

THIS volume like its predecessors is chiefly designed to assist in the transition from textbook to original papers. The aim has been to provide not a manual for the specialist, but a connected account based on the work of the last few years which may serve as a guide to current publications. Some attempt has been made to preserve a balance between the claims of cellular physiology on the one hand and the major systems whose interactions and responses provide the chief problems in the study of the organism as a whole. In selecting topics for inclusion it has been necessary to exclude biochemical, pharmacological and clinical matters, and also some subjects which have been extensively reviewed recently. The choice of material has presented a perplexing question and the omissions in a volume of this nature may appear in a different light according to the interests of the reader.

" Who ever saw one physician approve of another's prescription without taking something away or adding something to it? "

The diversity and expansion of physiological studies during the last decade have made it fitting that a number of authors should co-operate to produce the eighth edition of Recent Advances. This has encouraged a variety of approach, and writers have not been dissuaded from expressing personal opinions when dealing with controversial issues.

This volume has been made possible by the help of authors who have contributed chapters in spite of other pressing tasks. Their co-operation and their tolerance are very greatly appreciated by the editor. This opportunity may also be taken to acknowledge the generous help of several colleagues who have read parts of the script and have suggested improvements. The illustrations have been reproduced by kind permission of the original authors and publishers, and the source of these is given in the text.

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CONTENTS

Chapter 1

PERMEABILITY

by W. F. WIDDAS

Introduction

Many physiological processes depend on permeability changes and several important drugs and hormones are now known to exert their pharmacological action by affecting the rates of penetration of certain molecules or ions into cells. A full understanding of such phenomena will only be possible when the manner in which molecules and ions penetrate biological membranes is known. This is the central problem in permeability.

The earliest studies were with plant cells, but these were extended to unicellular organisms and cells of animal origin such as erythrocytes. A correlation was observed between rates of penetration and solubility in oily or lipid solvents. From a comparison of the penetration rates of many non-electrolytes the "lipoid theory of cell permeability" was advanced (Overton, 1895) for plant cells and supported for erythrocytes by Hedin (1897). The evidence suggested that, in permeating into cells, molecules had to cross a lipid barrier and that those molecules which were more soluble in lipids penetrated the fastest.

About twenty years later, in his " Principles of General Physiology ", Bayliss (1915) gave three possible modes of penetration which may be stated as follows:

1. Penetration through pores of a sieve-like membrane.
2. Penetration by solution in the substance of which the membrane is composed.
3. Penetration by the formation of reversible chemical compounds with substances of the membrane to which it is permeable.

Bayliss continued " The two last cases need not delay us long at this stage ". The shift in emphasis which had tended to favour the first mode of penetration, i.e. that based on the sieve hypothesis, was probably due to the greater preoccupation with the penetration of salts and certain similarities between biological membranes and artificial membranes which undoubtedly had sieve-like character-

istics. Chapter V of Bayliss's classical work is an excellent summary of the position at that time.

The developments in the three decades which followed included a widening of the field of study and the introduction of more precise techniques. The large volume of collected data supported both the factor of lipid solubility and the dependence on molecular size which was one of the characteristics in favour of a sieve-like structure. A membrane made up of a mosaic of lipid and protein was visualised and a 'lipoid-pore' theory adumbrated chiefly by Höber.

The first analytic approach to membrane permeability was made by Danielli on the basis that penetration was a process of activated diffusion. An important concept introduced into this analysis was that molecules required an activation energy to penetrate the membrane. This energy was necessary for the translation of the molecules and also to break down the hydrogen bonds with which the molecules were associated with other molecules in the water phase. In the case of many water-soluble molecules the latter formed the major part of the activation energy.

This stage in the progress of the subject has been fully documented by Davson and Danielli (1943), Höber (1947) and by Davson (1952). The first two modes of penetration and the compromise in the 'lipoid-pore theory' appeared to fit many examples of passive permeability. The physico-chemical principles were not different from those applicable to inanimate as well as living membranes and as such offered little new in the interpretation of physiological or pharmacological phenomena, although they had suggested that non-specific narcosis could be a membrane permeability effect.

During the last two decades the progress in this field, which most cogently brings Permeability into Recent Advances in Physiology for the first time, has been the recognition and study of cell permeabilities of the third type referred to by Bayliss. This comprises permeabilities in which there is good evidence of a reaction by the penetrating substance with components of the membrane. There are already many publications on these newer aspects of permeability and excellent reviews are available (Hodgkin 1951, Ussing 1960, Wilbrandt and Rosenberg 1961) for workers in this field. The purpose here will be to select topics which illustrate the trend of ideas and to introduce the concepts in a non-specialised manner. In this section the experimental details have been largely omitted so that attention might be directed to the underlying concepts. Mathematical formulae have been included to indicate the nature of the theoretical conclusions, but the derivation has in most cases not

been given here and for this purpose the reader should consult the original papers or refer in the case of well-known results to a standard work.

Nature of Cell Membranes

The early evidence of the lipid nature of cell membranes has been fully corroborated by later techniques such as polarisation optics, X-ray diffraction and high resolution electron-microscopy. Since membranes contain large numbers of different molecules the precise orientation must represent a rather unusual combination of intermolecular forces. The typical lipid molecule has one or more long

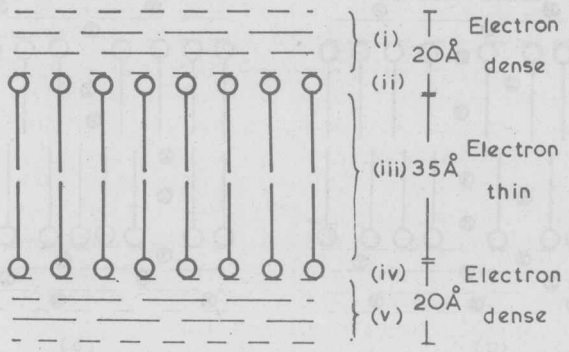

FIG. I.1. Schematic diagram of a cell membrane indicating the five layers which may be recognisable in an electronmicrograph. Layers (i) and (v) protein, layers (ii) and (iv) the polar head groups of the lipides, layer (iii) the paraffin chains of the lipides. When only three layers are resolved these are as indicated on the right.

paraffin-type chains with a "head-group" which usually contains all the water-attracting (polar) groupings. In an air-water surface such molecules tend to form insoluble mono-molecular layers with the polar head in the water and the paraffin chains (insoluble in water) standing out of the surface. The weaker attractive forces between the non-polar chains (Van der Waal's forces) help in stabilising this arrangement. In cell membranes the lipids are arranged in a bi-molecular layer, but electron-microscopists identify three and sometimes five layers of different density in an electronmicrograph. These are illustrated in Fig. 1 and comprise (i) an outer protein layer stabilising the array of polar head groups of the lipids, (ii) the outer

layer of "polar" head groups, (iii) the layer of non-polar chains (this region, although double, appears homogeneous in the electron microscope), (iv) the inner layer of 'polar' head groups and (v) the inner layer of protein.

Proteins formed into artificial membranes do not offer resistance to free diffusion of the type displayed by cell membranes and the permeability barrier must therefore be the double layer of lipid molecules. It should be realised that the electron microscopic

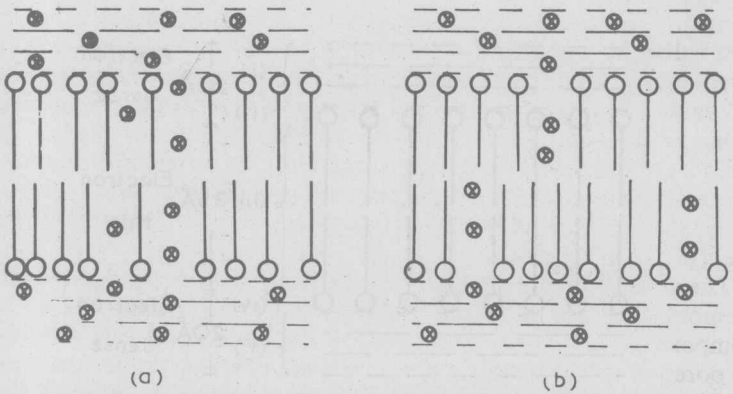

FIG. I.2. Schematic diagram to indicate the effect of thermal agitation in the plane of the membrane. Two possible instantaneous arrangements of molecules in a dynamic membrane are indicated by (a) and (b). In (a) a temporary pore extends right through the membrane; in (b) a pore does not exist but molecules may enter the lipid phase.

appearance is that of a fixed specimen and that in life the membrane is a site of vigorous dynamic action due to Brownian movement. This thermal agitation must be considered in two directions; firstly there may be an oscillatory movement of molecules in the plane of the membrane, and a possible consequence of such movement (depicted schematically in Fig. 2) is considered in the next section. Secondly, thermal agitation may result in movement of molecules between layers (ii) and (iv) in Fig. 1. That certain molecules with special affinities for complexing with sugars or salts may cross in such a way is a basic requirement of the hypothesis of a carrier transfer, which will be discussed in later sections.

Non-electrolyte Penetration

With the above in mind, it will be seen that penetration through a membrane by a small molecule will require (i) energy to leave the water phase and enter the lipid layer and (ii) the creation, or random occurrence, of a gap between the head groups and between non-polar chains into which the molecule can move. These two factors can be seen to be of different incidence. For example, (i) depends on the molecule itself and, as already pointed out, a large proportion of the energy required for penetration is often due to the necessity of breaking down strong inter-molecular attractions between polar groups of the molecule and water molecules (through hydrogen bonds). The energy of a hydrogen bond may be of the order of 5 K cal/mole, and thus molecules with several polar groups need a large excess of energy to break away. This limitation also applies to evaporation and it is everyday experience that solutes with strong polar attractions do not easily escape into the vapour phase. In contrast, solutes which are sufficiently non-polar to enter the vapour above a watery solution penetrate cell membranes more readily. Often such substances have characteristic odours which substantiate their presence as vapour.

The second requirement (ii) is more dependent on the state of the membrane and possibly the mobility and thermal agitation of its components. A lateral oscillation of lipid molecules will give rise to pores or gaps between the head groups of varying size, and thus the random occurrence of gaps will favour molecules of small size (which can use large or small gaps) and could be responsible for the sieve-like characteristics. The relative importance of factors (i) and (ii) may be expected to vary in cell membranes of different species.

The quantitative analysis used by Danielli was based on the concept of a number of potential energy barriers (see Appendix A in Davson & Danielli, 1943, and Fig. 3). Thus there was a potential energy barrier to be crossed on entering the membrane (with rate constant a), one on leaving the membrane (with rate constant b) and a number (n) of smaller barriers in the membrane (with rate constants e). It was shown that for slowly penetrating molecules the permeability would be chiefly determined by a, the rate constant for leaving the water phase and entering the membrane, whereas, for more rapidly penetrating substances, the permeability depended on a term including a/b which was identical with the oil/water partition. The use of Danielli's analysis in describing permeability data proved of value in comparing the penetration rates of various compounds and drawing attention to molecules whose penetration appeared to

be many times faster than expected from such a physico-chemical approach.

In recent years a new analytical approach to the penetration of small lipid insoluble molecules has been made on the lines of a sieve-like membrane. The analysis of rapidly penetrating substances usually involves an osmotically induced volume change which is the quantity measured. Where water penetrates so much faster than the

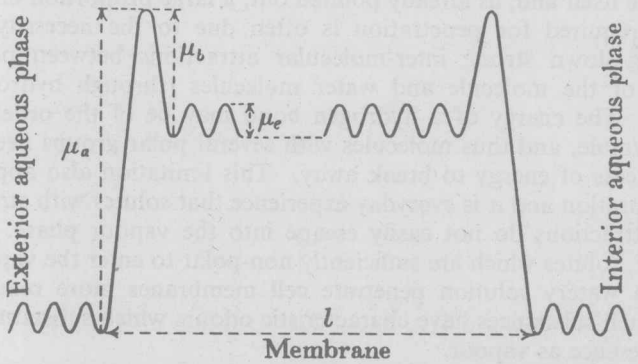

FIG. I.3. The potential energy barriers proposed by Danielli. These include (i) the potential energy barrier (μ_a) between water and membrane phase, the rate constant for which is a, (ii) small energy barriers in the membrane, each with rate constant e, and (iii) the barrier from the membrane back into the water phase, with rate constant b. As the process is symmetrical, the barriers for which a and b are appropriate are shown at both sides of the membrane.

solute, the assumption of continuous osmotic equilibrium can be made and changes in volume can be used to deduce changes in internal concentrations of the test substance. If solute and water are permeating at comparable rates this approach is invalid, but a new approach based on irreversible thermodynamics (Staverman, 1951) has been used (Kedem & Katchalsky, 1958). The parameter which is found useful in this analytical approach is Staverman's reflection coefficient σ. If the expected osmotic pressure for a solution of a test molecule is πTHEOR = RTC (R is the gas constant, T the absolute temperature and C the concentration) and the observed osmotic pressure is, as obtained from the concentration needed to prevent initial water movement, πOBS, then the reflection coefficient (σ) is given by

$$\sigma = \frac{\pi\text{OBS}}{\pi\text{THEOR}}.$$

For a membrane totally impermeable to the test solute $\sigma = 1$ and for the case of a solute which penetrates as fast as water $\sigma = 0$. If a sieve-like structure is assumed it is possible to deduce an equivalent pore radius from the reflection coefficients determined for a number of (lipid-insoluble) molecules of different molecular diameter. For instance, Goldstein and Solomon (1960) arrived at an equivalent pore radius for human red cells of 4·2Å and Table 1 summarises their data and results.

TABLE 1

Data by Goldstein and Solomon (1960) *for human erythrocytes*

	Mean iso-osmolar concentration	σ	$1 - \sigma$	Mean molecular radius Å	oil/water partition $\times 10^3$
Glycerol ..	0·33	0·88	0·12	2·74	0·07
Propylene Glycol	0·34	0·85	0·15	2·61	5·7
Malonamide ..	0·35	0·83	0·17	2·57	0·08
Methyl urea ..	0·36	0·80	0·20	2·37	0·44
Propionamide ..	0·36	0·80	0·20	2·31	3·6
Acetamide ..	0·50	0·58	0·42	2·27	0·83
Ethylene Glycol	0·46	0·63	0·370	2·24	0·49
Thiourea ..	0·34	0·85	0·150	2·18	1·37
Urea	0·47	0·62	0·38	2·03	0·15

The mean iso-osmolar concentration is that in which the cells would neither shrink nor swell for a brief initial period and is obtained by an extrapolation procedure. σ is the reflection coefficient. The area available for solute diffusion relative to that for water is given by $(1 - \sigma)$ and should correlate inversely with a function of the molecular radius. The magnitude of the oil/water partition coefficient is shown in the last column.

The apparent areas for filtration of solute relative to water may be related to σ as follows:

$$1 - \sigma = \frac{A_{SF}}{A_{SW}}$$

where A_{SF} is the area available for solute filtration and A_{SW} is the area available for water. One would expect this ratio to be lower for substances of larger molecular radius and vice versa. It will be seen that the data in Table I does show such a trend, but the values for acetamide and thiourea are anomalous. Moreover, it is necessary to stipulate that lipid soluble molecules can enter by a separate route and the analysis does not distinguish between a permanent pore structure and a temporary structure in a continually changing dynamic membrane.

The use of irreversible thermodynamics may be regarded as an important new method of analysing permeability results where osmotic equilibrium cannot be assumed, but it has not resolved the problem as to whether the membrane can be regarded as essentially homogeneous or whether discrete water-filled channels are present as a permanent feature.

Physico-chemical Properties of Sugars and Ions

Analysis along the above lines has proved inadequate to account for the permeability characteristics of cells towards sugars and inorganic ions. This is probably related to a common physico-chemical feature shared by sugars and ions, namely their strong association with water molecules. In the case of sugars the free —OH groups associate with water molecules through hydrogen bonds, whereas with the ions the strong electric field has the effect of orientating and holding a shell of water molecules (because of their own dipoles) around each ion. The electric field from a point charge varies inversely as the radius, and small ions such as Li^+ and Na^+ hold a larger shell of water than K^+ or Rubidium ions. The field is also increased for divalent ions such as Mg^{++} and Ca^{++}.

Table II illustrates the water-binding power of different polar groupings and also gives for comparison the ionic diameters in crystals (water free) and in solution (hydrated) of some biologically important ions.

Neither sugars nor salts enter the vapour phase during evaporation and generally they possess those qualities which militate against the penetration of a homogeneous lipid membrane. The fact that these important substances in cell metabolism and physiology do penetrate cells may be thought to be in favour of the sieve hypothesis, but for several reasons specialised transfer mechanism for these substances have been postulated. The nature of the mechanism in detail is still unknown and there have been many speculations. The hypothesis which has proved most fruitful in interpreting the various permeability phenomena, and in suggesting lines for further research, has been that of a " membrane carrier ". The application of this concept to the permeability of sugars and inorganic ions will be described in the following sections.

Permeability to Sugars

Single cell preparations can most conveniently be used for determining the rates of sugar penetration and most of the kinetic analysis of the process has been carried out with the human erythrocyte.

TABLE II

Polar groups in non-electrolytes which attract water molecules

Group	*No. of water molecules associated*
H_2O	4
$-OH$	3
$-COOH$	4—5
$=O$	2
$-NH_2$	3
$=NH$	2
$=N-$	1

Ionic binding of water

Ion	*Crystal diameter*	*Hydrated diameter*
	Å	Å
Li^+	1·2	4·6
Na^+	1·9	3·56
K^+	2·66	2·44
NH_4^+	2·96	2·42
Rb^+	2·96	2·36
Cs^+	3·38	2·32

The hydrated diameters are derived from the diffusional mobilities of the ions. They are less accurate than the crystal diameters but show that the smallest ions hold the largest shell of water molecules when in solution.

It is known that glucose penetrates human red cells until the internal and external concentrations become equal. As long ago as 1897 however, Hedin showed that the cells were not permeable to the isomeric inositol, and Kozawa (1914) showed that monosaccharides penetrated at different rates. Ege (1919) showed that the rate of penetration of sugar was less at high concentrations. Thus forty years ago features of sugar permeability were known which could not be explained on a basis of sieving by molecular size.

The newer work in this field was based chiefly on the discovery (Ørskov, 1935) that volume changes in red cells (which fell short of producing haemolysis) could be followed by passing a beam of light through a suspension of cells and recording changes in its intensity with a photo-electric device. This fact, coupled with the slow penetration of sugars relative to water (and the assumption of osmotic equilibrium), has made it possible to follow continuously the variations in the internal concentration of the cells and so to measure rates of penetration.

For an " entry " experiment a strong solution of sugar in saline is added to a suspension of cells. The cells first shrink as they lose water to attain osmotic equilibrium and then, as the sugar penetrates, they slowly return to their original volume. An example of the light changes measured photo-electrically and recorded by an ink-writing milliammeter is illustrated on the right-hand side of Figure 4. In a typical " exit " experiment the cells would first be

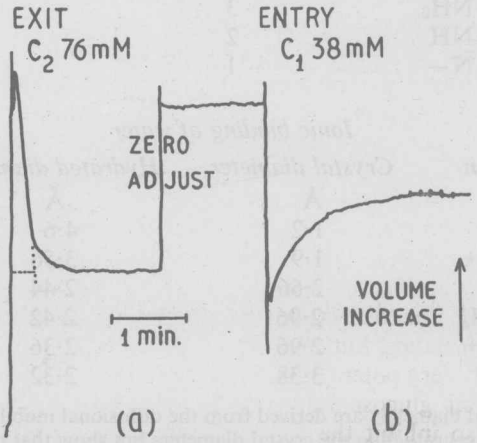

FIG. I.4. Records from a photoelectric apparatus during glucose " exit " and " entry " experiments with human erythrocytes. (a) The cells were initially incubated with 76 mM glucose and, on addition to glucose-free saline, they first increased in volume due to an osmotic entry of water; during the " exit ", glucose and water were lost from the cells which returned to their isotonic volume. (b) On addition of glucose solution to bring the medium up to 38 mM the cells first shrank from loss of water, but returned to their isotonic volume as glucose penetrated the cells. There is a difference in final light transmission due to the dilution of the suspension by the added glucose solution.

equilibrated in saline containing a known concentration of sugar and an appropriate quantity of cells added to a large volume of sugar-free saline. Under these circumstances the cells first swell by taking in water osmotically (due to the extra sugar inside), then, as the sugar leaves the cell, they also lose the excess water and return to their initial volume. An " exit " record is shown on the left-hand side of Figure 4.

When the sugar is glucose, the records of " entry " and " exit ", as in Figure 4, have quite different characteristics which could not be explained if the process were one of simple diffusion through