THE PERMEABILITY OF LIVING CELLS

S. C. BROOKS
AND
MATILDA MOLDENHAUER BROOKS

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PREFACE

In writing this monograph we are reminded of the parable of the Joyous Young Man who set out to conquer the world. As Time proceeded swiftly onwards, less and less of the far countries were included in his scope, even fewer and fewer of the outlying districts. He restricted his endeavors more and more, and finally learned that if he would hold his own in his own native district, that was as much as was permitted in his brief life-span to conquer.

In compiling the material in connection with this monograph, it soon became evident that our original plans for making this a comprehensive study of all the factors involved in the study of Permeability, would have to be modified. Indeed, only a fraction of what we desired to present has finally come out of our past hopes. And so we have become convinced that the study of Permeability might be synonymous with the study of Life itself and those factors which determine whether or not a cell survives; that the Unknowns far exceed the Knowns; that the Knowns have led into many fields of Science which are closely related to Biology, Physics, Chemistry, Biochemistry, Anatomy, Physiology, Mathematics and Medicine, but that one can at best pluck only a few of the truths and observations which it has taken thousands of workers to produce.

And so we have finally attempted to crystallize into a critique a few of the factors which we have deemed important in connection with the present-time experimental inquiry into Permeability.

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S. C. Brooks
M. M. Brooks

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ADDENDA

Since writing this volume, the exigencies of the War have considerably delayed its publication and meanwhile new methods have added a fund of new facts.

Owing to the impossibility of including a complete bibliography of the past few years only a brief mention of the most important of these experiments from the point of view of ion permeability will be made. These deal with the use of radioactive elements, artificially produced, which have brought to light some surprising conclusions. A first approximation that the speed of penetration of these ions is nearly equal to the rate of free diffusion was discussed by S. C. BROOKS (1937). Since that time confirmation of these results has been found in further experiments not only by S. C. BROOKS (1939a, 1939b, 1940a, b) but by others using different cells.

In order to see if there were any effects upon the metabolism of these cells by the radioactive elements, M. M. BROOKS (1939) used the WARBURG-BARCROFT respiration apparatus and studied the oxygen consumption of *Nitella*, *Elodea* and various red blood cells. It was found that radioactive NaCl and KCl whose activities ranged from 2.2 mC/liter to 20 mC/liter had no appreciable effect as determined by these methods. MULLINS (1939) investigated the Na-radioactivity of Na*Cl only, by placing it in small glass bulbs immersed in the solution containing *Nitella*. He found that above 1.0 mC/liter there is a decreased rate of penetration of the ions but below this there is no effect. Since it would require extensive treatment to discuss all of these results only a few references will be made here. One important conclusion from the experiments relates to a long-standing controversy as to whether substances penetrate cells as molecules or ions. It had been generally agreed that molecules penetrate cells as molecules, but the question of

ion penetration was left open. The experiments with radioactive ions leave no doubt that ions penetrate by ion exchange, and contrary to the old belief, in minutes or seconds, although in some cases they enter slowly, viz., in several hours. Previous results were dependent upon limitations of the then-existent methods. Since this volume is also in the nature of a critique of the older literature, the tables taken from the experiments produced by the older methods will be left in the volume, hoping that some historical value will be found for them. The reader is referred to the Conference on Applied Nuclear Physics, Cambridge, Mass., October 28 — November 2, 1940, published by the Massachusetts Institute of Technology. This outline contains short abstracts and references to most of the present-day experiments in radioactive elements in relation to permeability. Most of the work deals with the movement of tracer elements into mammalian tissues. Brief mention may be made of the work of HAMILTON and SOLEY on the distribution of iodine in humans, and that of HODGE, MANN and ARIEL, in rabbits; the distribution of potassium, sodium and chlorine in rats and rabbits by FENN (a similar study was made of tissue distribution of sodium by GREENBERG, CAMPBELL and MURAYAMA, 1940); of sodium and potassium in red blood cells by COHN; by WINKLER, EISENMANN and SMITH, and by LARK-HOROWITZ; the distribution of calcium and manganese by GREENBERG; the uptake of phosphorous by tissue cells by BRUES, JACKSON and COHN; the penetration of inorganic arsenic into red blood cells, by HUNTER and KIP; the location of the upward movement of mineral salts in plants by GUSTAFSON and DARKEN, and BENNETT, STOUT and HOAGLAND; and a recapitulation of the ion movement in living protoplasm by S. C. BROOKS.

BROOKS, M. M., 1939. Effect of certain radioactive elements on the metabolism of cells. Proc. Soc. Exptl. Biol. Med. **42**, 558—59.

BROOKS, S. C., 1939. Ion exchanges in accumulation and loss of certain ions by the living protoplasm of *Nitella*. J. Cellular Comp. Physiol. **14**, 383—401.

BROOKS, S. C., 1939. Intake and loss of radioactive cations by certain marine eggs. Proc. Soc. Exptl. Biol. Med. **42**, 557—58.

BROOKS, S. C., 1940. *Ion intake by living cells.* Biol. Bull. **79**, 341—42.

GREENBERG, DAVID M., W. WESLEY CAMPBELL and MAKIO MURAYAMA, 1940. Studies in mineral metabolism with the aid of artificial radioactive isotopes. V. The absorption, excretion, and distribution of labeled sodium in rats maintained on normal and low sodium diets. J. Biol. Chem. **136**, 35—46.

MULLINS, LORIN J., 1939. The effect of radiation from radioactive indicator on the penetration of ions into *Nitella.* J. Cellular Comp. Physiol. **14**, 493—5.

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INTRODUCTION

In order that the various substances which compose a living cell should be properly distributed throughout the cell, it is essential that they pass inwards through the surface of the cell and through the protoplasm. In the same way, metabolic reagents and waste products must pass inwards or outwards through the cell surface and protoplasm.

The rate at which this transport of materials is to occur will depend upon two factors: the driving force, and the resistance which particular parts of the cell offer to the movement of the material in question. It is the latter factor which, strictly speaking, should be referred to as permeability, but both factors must be taken into account if we are to obtain a clear picture of the mechanism by which import and export materials are determined in living cells.

Permeability may be defined as the rate of movement of a substance through the permeable layer under a given driving force. This definition involves two concepts which must be kept sharply distinguished: on the one hand, permeability, — a property of a membrane or region of the cell: on the other hand, the driving force which may be quite independent of any property of the membrane. To these two concepts correspond two others, which must similarly be clearly separated in our treatment. Driving force measures how far short of equilibrium the system is: at equilibrium for any given molecular species the driving force for that species must be zero. Permeability measures the rate at which equilibrium is approached, that is, what fraction of the total change necessary for the attainment of equilibrium is accomplished in unit time.

Plasma and other semi-permeable membranes

The question immediately arises as to where in the cell the semipermeable layers are located. Is there, for instance, a plasma membrane, forming the outermost layer of living cells, and responsible for the typical permeability relations of the cell? Are nuclei and vacuoles surrounded by similar membranes? Or, on the other hand, does the cytoplasm or nucleoplasm have equal permeability throughout its whole volume? Without presenting detailed evidence in this place (See e.g., Chapter I, by CHAMBERS, in Harrow & Sherwin) we may say that there is good reason to conclude that at least some of the interfaces of living cells (e. g., superficial and vacuolar) are considerably less permeable than the bulk of the protoplasm, and usually dominate in determining the normal activities of cells and the outcome of experiments. The influence of the permeability of various parts of the cell other than interfaces, may usually, though possibly not always, be neglected. We shall, therefore, assume, in the absence of evidence to the contrary, that the interfaces at the outer boundary of the cytoplasm and between at least some of the various portions of the living cell, are the seats of characteristic permeability and may be thought of as semi-permeable membranes. Only such living membranes, for which we shall use the general term "protoplasmic membranes", about whose permeability we have any considerable knowledge are the superficial or plasma membrane and the vacuolar and nuclear membranes. A complete picture of the permeability relations of organisms or even of many cells must include excreted and apparently non-living membranes, such as plant cell walls, animal cuticle and the like.

Driving forces:

Activity gradients and electrical forces. The underlying cause of the movement of solutes and water in living organisms is undoubtedly concentration gradients, or, more properly, activity gradients. Secondarily, the activity gradient of one substance may give rise to an electrical potential gradient, which in turn may act as a driving force upon some other substance. Therefore, in considering the forces acting to drive a given substance through a membrane we must include not only its own activity gradient but also the possible effects of potential gradients across

the membrane. These two forces (besides mechanical movement of the solution as a whole) seem to be the only ones possible.

Activity gradients. Substances in solution do not necessarily behave as would be predicted on the basis of their stoichiometric concentration, but display properties proportional to the activity (LEWIS, 1907)[1]. Activity has no dimensions and is proportional to a fugacity ratio as shown by the equation $a = f/f^0$ in which f^0 is the fugacity of the substance in a chosen standard state. Since f^0 by definition is equal to one, the activity, a, is numerically equal to the fugacity, f, of this equation. The fugacity measures the tendency of a substance to escape from the phase in which it is.

In the case of a substance in solution, the fugacity is the tendency of that substance to leave the solution. Fugacity has the dimensions of a force, and it may be measured by such properties as vapor pressure, correction being made for the fact that the vapor is an imperfect gas (LEWIS and RANDALL, 1923). Since there is a direct relation between fugacity and molal free energy, any measure of the latter will serve as a measure of differences in fugacity. Measurement of electrode potentials is an example. Since the equation connecting partial molal free energy, $\bar{F}$, and fugacity, f, has the form

$$\bar{F} = RT \ln f + B,$$

where B is a function of temperature only, it is possible to obtain from the known values of $\bar{F}$ the *ratios* of f in two different systems, A and B, since $F_B - F_A = RT \ln \frac{f_B}{f_A}$.

The activity of any substance in a selected standard state is, by definition, equal to unity. In the case of water it is more convenient to choose pure water at the given temperature and external pressure as the reference state, and to assign arbitrarily to water in this state an activity of one. For solutes other standard states have been used. For non-electrolytes the standard state is that state at infinite dilution, while for strong electrolytes the state is that concentration at which, for example, a univalent salt yields equality of the product of the ion activities and that of the undissociated molecules. This is expressed by the equation.

[1] See also LEWIS and RANDALL (1923), especially Chapters 17 and 22.

$a_+ \cdot a_- = a_2$, where a_+, a_- and a_2 refer to the cation, the anion and the undissociated molecule respectively. The quotient $\frac{a}{c}$ is the activity coefficient, γ, of the ion in question.

Either fugacity or activity may be used to express the force which produces diffusion. Diffusion underlies the movement of water and solutes. Concepts of activities, fugacities and activity or fugacity gradients are of general applicability, and as such, may be used to unify, under one method of treatment, the first and most fundamental type of driving force which results in the passage of materials through protoplasm. Thus, when water passes through a membrane from a dilute to a concentrated solution, it is in reality moving from a region of higher activity to one of lower activity. A solute diffusing through a membrane from a concentrated to a dilute solution is also moving from a region of higher activity to one of lower activity. In the first case, the movement of water is generally known as osmosis, and although not all physical chemists take this view, it seems best to us to apply the term osmosis to the essentially similar movement of a solute when it diffuses through a membrane. The distinction between solvent and solute is at best artificial.

Whenever water or dissolved substances pass through a membrane along their activity gradients we shall, therefore, speak of *osmosis of water or dissolved substances*, and of *endosmosis* or *exosmosis* according as the water or solute is passing into or out of the cell.

In order to express the permeability quantitatively, it seems desirable to reduce the activities to a common basis for solutes, molecules and ions. We can do this by using a suitable term to express activities in terms of molality as corrected by the activity. This is discussed further elsewhere.

There is another effect of simultaneous movement of solute and solvent on each other's rate of movement. The effect discussed in the previous paragraphs is one which affects the driving forces, and is proportionate at any moment to the activity gradients existing at that moment. In addition it is also probable that the rate of movement of one substance affects the simultaneous rate of movement of all other substances in a given solution. To take an extreme case, let us imagine a narrow cylinder containing a sugar solution whose concentration increases regularly from a point A to