TRANSPORT and ACCUMULATION in BIOLOGICAL SYSTEMS

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FOREWORD

This volume represents the result of an attempt to gather together some of the extensive amount of information which has been published more recently on the subjects of cellular inorganic chemistry and the kinetics of movement of inorganic and simple organic substances between cells and their surroundings. The advent of radioactive tracers and the development of flame photometry as an analytical tool accounts for the progress in the field of cell permeability, but the mechanism of the nervous impulse has been elucidated by quite different methods with results of fundamental importance.

Almost any process involving cells and substances can be formulated in terms of permeability or adsorption coefficients, so little excuse is needed for limiting the subject matter to selected examples. References to the literature have mainly been to later work, for such papers themselves provide earlier references when these are required. No attempt is made to give credit for first discoveries, and such important papers as have been omitted represent gaps in the author's capacity to find or summarize all relevant material.

E. J. HARRIS

ACKNOWLEDGMENTS

It is an agreeable duty to thank here all my friends at University College, London, who have helped in various ways to make this work possible. In particular Professor B. Katz, F.R.S., for letting me spend the time and for reading the draft, and to Doctors B. Ginsborg and C. Edwards for making numerous suggestions and corrections. Professor H. B. Steinbach has kindly helped with proof reading and Doctors G. Eggleton and H. Davson have given helpful advice as a result of which the number of mistakes has been reduced. Mr. Marmoy and his assistants at the Library have been most kind and have saved me much time in obtaining various sources of information.

It is also a pleasure to record that my colleagues throughout the world have freely allowed use to be made of their illustrations. Reference to the appropriate author(s) is made in the captions.

INTRODUCTION

A BIOLOGICAL system can be regarded as being built up of cells and of cellular secretions. The chemical constitution of the cells is widely variable, and when it is possible to examine the separate histological components of the cell each is found to possess its own chemical individuality. The cells usually grow in a medium which is of different composition from that of the cells. It is commonly inferred that some sort of boundary or limiting membrane separates the cell contents from the medium and the microstructures within the cell from the cytoplasm. In some cases (e.g. the erythrocyte) the membrane can be detached, but in other cases the evidence depends on electron microscopy.

When certain specialized cells form an organized structure the assembly can acquire the property of moving some substances in a particular direction. Among such secreting layers are the kidney tubules, the wall of the intestine and certain skins.

The subject of permeability covers the examination of the rate, and mechanism, of passage of chemical substances through any boundary, be it cell wall or sheet of cells. Obviously permeability controls the rate of ingress of nutrients and the egress of waste products. We shall examine the composition of the components of some cells in relation to that of the medium to see how much accords with the operation of physicochemical laws. We shall discuss the behaviour of some secreting layers and see what is known about the operation of the chemical forces which move materials selectively in one sense. When these latter forces act on a substance its distribution does not follow simple physicochemical laws and instead is linked with the progress of a metabolic reaction. Let us consider the well-known fact that many animal and vegetable cells maintain a high K and low Na content. This might have been explained by these ions being shut off in an impermeable compartment, but tracer studies have revealed that most, if not all, of the total ion content will undergo exchange with labelled ions in the external medium. This shows that there is a continuous inflow (influx) and outflow (efflux) of these ions. Electrical studies show that the K distribution is often not far removed from that expected on physicochemical grounds, whereas the Na distribution is quite the contrary and a chemical mechanism must be invoked. The biennial reviews on "Transport through Biological Membranes", published by the Annual Reviews of Physiology contain useful summaries of the subject-matter partly covered in Chapters 5–12 (WILDE, W. S. (1955). "Transport through Biological Membranes", *Annu. Rev. Physiol.* **17**, 17–36).

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MEMBRANE STRUCTURE AND PENETRATION

PASSAGE of molecules or ions from a solution into a membrane when no chemical forces operate is the result of the collisions which are going on continually between the particles and the membrane due to thermal agitation. When in the membrane the particle is still subject to agitation and it may eventually be expelled on one or other side. This is an example of the operation of diffusion; diffusion constants for substances in a membrane usually differ from those in aqueous media. There are several ways in which the substance can be distributed while it is within the membrane. It can be in the form of a solution, being dissolved in the membrane material, or it may only be able to pass through the aqueous solution filling the pores or channels between the molecules making up the membrane. When the channels are of molecular dimensions the only difference between this mode of passage and the homogeneous process is that the penetrating molecules or ions are kept in the one channel instead of being free to pass in any direction between membrane molecules. The rates of both types of penetration will be determined by a combination of steric and electrical factors.

A third mechanism for transferring a particle to the other side of a membrane is by a combination with some substance at one surface, followed by traversel of the membrane in a combined state, and liberation at the other side by a second chemical reaction. It has proved difficult to obtain much information about the structural features involved, or the chemistry of such processes. The following paragraphs will deal with membrane structure and aspects of physical transfer of substance through membranes.

THE ERYTHROCYTE MEMBRANE

As the membrane of the erythrocyte is readily obtainable it has been studied extensively by both physical and chemical means. The membrane or "ghost" is prepared by treating the cells with some agent such as a surface-active compound, or a hypotonic solution, this causes them to lose haemoglobin. The preparation has been described by PARPART (1942). The membrane material contains

both lipid and protein; in ox cell stroma the weight ratio is 1 : 1·76 (PARPART and BALLENTINE, 1952a and b). The main fibrous protein component makes up 4 per cent of the total protein. It is called stromatin, its properties are discussed by JORPES (1932) and BALLENTINE (1944). Other constituents, of protein and lipoprotein nature, have been described by MOSKOWITZ and CALVIN (1952). In the stroma of ox cells the lipid fraction (equal to one-fifth of the dry weight) includes cholesterol (30 per cent), cephalins (46 per cent), lecithins (11 per cent) and sphingomyelin (8 per cent) (and 5 per cent unaccounted). A proportion of the lipids is more readily removed by fat solvents than the rest, so it appears that there are different states of combination between lipid and protein.

The recovery of about 1 per cent of the original cell weight as membrane material, together with estimates of the surface area per cell and the number of cells per unit weight, permits the calculation of the membrane thickness. The result is 52Å, which agrees with a value found using the electron microscope. It is lower than a figure (200Å) found by a different technique in which the reflectivity of cell ghosts deposited on a polished metal plate was compared with that of known thicknesses of barium stearate film (the leptoscope, described by WAUGH and SCHMITT, 1940) .

The difference in result between the electron microscope and the leptoscope has been said to be due to haemoglobin caught beneath the membrane in the preparation of the leptoscope films, but other techniques, too, have suggested that in the natural state the membrane is thicker than 50Å. In particular it must be recalled that when wet the protein will be hydrated and probably much more swollen than after the drying necessary for electron microscopy. Ponder has discussed the problem. The volume occupied by ghosts is between one-half to one-twentieth of the volume of the cells according to the degree of washing. From this the cell wall could be as thick as 1000Å, but there might be liquid included between much thinner walls. However, fragmented ghosts occupy 80 per cent of the volume of unbroken ones, so there cannot be a great proportion of fluid included. The difficulty in attaining a complete separation of cell lipid from the protein led Ponder to propose that there is a gradual variation ratio lipoprotein/protein from membrane to cell centre, with the ratio at its highest at the exterior. It is, however, possible to remove a great deal of the protein by haemolysis, leaving a residue mainly of lipoprotein. Evidence suggesting that this residue still contains some haemoglobin is provided by the cell ghosts prepared from the blood of sickle cell anaemic patients. The haemoglobin of these cells changes to a

semi-crystalline or tactoid form (PERUTZ, 1951) when the oxygen tension is reduced. When the test is applied to the cell ghosts these also reveal the presence of tactoid material.

The electrical properties of the red cell membrane have been measured. At a low frequency the cells in a suspension behave nearly as insulators, while at high frequency the membrane capacity permits the current to enter the interior and reveal the presence of a volume of conducting material within. HÖBER (references in his book 1945) was one of the pioneers in the use of the technique. It was applied by FRICKE (1933) to red cells and to the study of the changes produced when haemolysis occurred (FRICKE and CURTIS, 1935). The resistance of the membrane is about 1000 ohm cm^2, and the capacity is 1 $\mu F\ cm^{-2}$ at low frequency. Both values decline as the frequency is raised. The frequency dependence of the capacity was changed after haemolysis had released haemoglobin from the cells.

FRICKE (1925) estimated the thickness of the membrane as 33Å from the capacity, taking the dielectric constant as 3. The specific resistance of the cell contents was estimated to be 3·5 times that of the serum (FRICKE and MORSE, 1925). HILLIER and HOFFMAN (1952) in an electron microscope study of the dry cell membranes concluded that the thickness is 50Å. This was thought to be built of a layer of a fibrous material having 20Å diameter fibres each about 200Å long overlain by what they called "plaques" 30Å high and about 200Å diameter (see Plate I). The diameters of the plaques varied among cell samples drawn from different individuals. The plaques could be detached by treatment with ether so it was suggested that they are attached to the protein mesh by a lipid layer. They might be composed of a double thickness of lipoprotein with the lipid ends exposed.

EGG MEMBRANES

The possession of as many as three membranes has been attributed to some marine eggs. The outermost, called the vitelline layer, is soluble in 1 M urea (KOPAC, 1940). This layer protects the plasma membrane of the unfertilized egg. Upon fertilization or micropuncture there is secreted between the vitelline layer and the protoplasm a jelly called the "hyaline" layer, which may even lift off the vitelline membrane. This hyaline membrane is soluble in solutions of univalent salts (CHAMBERS, 1940) and the residual cell is still capable of division, though it is very sensitive to mechanical damage. COLE (1940) measured the resistance and capacity of egg membranes. As with red cells the capacity varies with

frequency. The values are changed when the eggs are fertilized. Common values are 1000 ohm cm^2 for resistance and 1 $\mu F\ cm^{-2}$ for the capacity.

CALCIUM IN MEMBRANES

The alkaline earth calcium seems to play an important part in the structure of a variety of biological membranes. The calcium content is one factor determining the permeability to water of the capillary wall, the erythrocyte membrane and *Arbacia* eggs. MAZIA (1940) examined the incorporation of calcium into a number of membranes; he found that part could not be extracted with distilled water. The non-extracted part, if expressed per unit area, did not vary greatly between yeast, *Elodea* and *Arbacia* eggs. The whole of the calcium could be removed with citrate, and subsequently a non-extractable (by water) fraction could be replaced on treatment with fresh calcium salt. If the cells are freed from Ca by treatment in citrate solution and are treated with ribonuclease before exposing them to a fresh supply of Ca ions they then fail to recover their Ca. This suggests that the Ca is taken up initially by combination with a surface layer of ribonucleic acid (LANSING and ROSENTHAL, 1952).

The specificity of the affinity of the cell wall of *Elodea* for calcium has been studied by using the cell sap to provide a convenient test for Ca in the cell wall (Mazia). The method depends upon the fact that the cell wall releases its Ca into the sap if the cell is first treated with a hypertonic solution to make the interior shrink away from the cellulose wall (that is, to plasmolyse) and is then replaced in an isotonic medium so that the interior swells again up to the confine of the wall. The sap contains oxalates so that released Ca forms a precipitate visible under the microscope. If the cells are washed in a Ca-free solution of Na, K or Mg chloride previous to plasmolysing and returning to normal there is no release of Ca, indicating that the Ca has exchanged for another ion. If mixtures of Ca and other ions are used the cell wall will retain Ca in a mixture of Na/Ca 200/1 or in Mg/Ca 20/1, indicating a greater affinity for Ca than for the other ions. In general the force acting on adsorbed ions will vary as the square of their valency, so this leads one to expect a difference between Ca and Na, but the difference between the divalent pair Mg and Ca must presumably arise because of a difference in size of the ions.

Calcium ions have been found by HEILBRUNN (references in his book, 1937) to be involved in the formation of the precipitate which covers protoplasm emerging from the interior of a damaged cell.

MEMBRANE PENETRATION

Penetration of a membrane may occur by several processes, namely

(*a*) by solution in the lipid,
(*b*) by passage through pores,
(*c*) by temporary combination with a membrane group,

or these processes may be concerned together.

(*a*) *Penetration by solution in the membrane*

Electrical measurements indicate that the cell membrane has a high resistance, so it has been proposed that the lipid forms a continuous layer, with perhaps a number of pores lined with ionized groups passing through it (DAVSON and DANIELLI, 1936; DANIELLI, 1952).

Table 1. Comparison of the permeability of *Chara* cells with oil/water distribution coefficients and diffusion constants of the diffusing substances.*

Substance	Permeability constant μ/sec	Oil/water ratio $\times 10^8$ r	Diffusion constant in membrane $\times 10^8$ cm^2/sec
Methanol	280	7·8	7·2
Ethylene glycol	12	0·49	4·9
Propylene glycol	24	5·7	0·84
Glycerol	0·21	0·07	0·60
Erythritol	0·014	0·03	0·093
Glycerol methyl ether	12	2·6	0·92
Glycerol ethyl ether	21	7·4	0·57

* The figures are calculated from various authors' results by ZWOLINSKI, EYRING and REESE (1949) assuming for the estimation of D that the wall thickness is 200Å and that the distance between the sites in the membrane (that is, the potential energy minima) is 5Å.

Non-polar substances would be expected to penetrate a lipid layer by dissolving in it. When the rates of transfer of various non-electrolytes into the plant cells *Chara* or *Nitella* were examined it was found that there is a rough correlation between the permeability constant P and the oil/water partition ratio r (see Table 1) (COLLANDER and BARLUND, 1933; COLLANDER, 1950). The absence of a strict proportionality can be ascribed to differing rates of diffusion in the membrane, for this as much as the solubility factor must influence the kinetics. If the dependence of the diffusion rate in the membrane resembled that holding for gaseous diffusion we should expect the product $PM^{0.5}$ to be constant for a given solubility, where M is the molecular weight of the diffusing particles.

Then for varying solubility $PM^{0\cdot5}$ should be linearly related with r. The graph in Figure 1 (due to COLLANDER) shows that no such exact relation holds, small molecules pass through the membrane faster than predicted, probably because they traverse the pores as well as passing through the lipid.

DANIELLI (1943) suggested that the potential energy curve of a particle as it passes through the membrane has a number of maximae

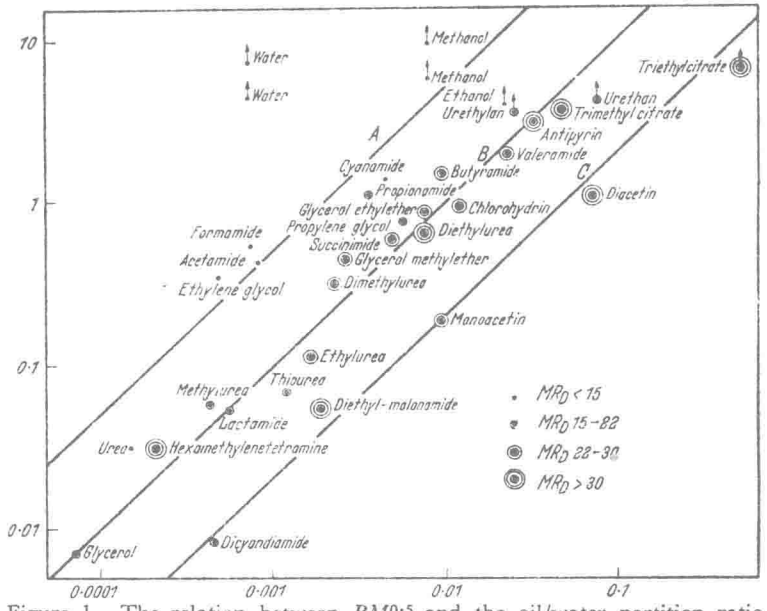

Figure 1. The relation between $PM^{0\cdot5}$ and the oil/water partition ratio for a number of substances crossing the membrane of *Chara* (COLLANDER, 1949: reproduced by courtesy of *Physiologia Plantarum*). MR_D is a measure of the molecular size: abscissa is partition rate: ordinate is $PM^{0\cdot5}$.

and minimae, as well as there being larger changes at the membrane boundaries (Figure 2). In this event the rate of diffusion will depend upon the energy difference E_m required to dislodge the particles from the minimae. This can be inferred from the temperature coefficient of the diffusion rate. According to the Boltzmann law the rate at which a mole of the particles will receive extra energy E_m will be proportional to $\exp(-E_m/RT)$, so if the ratio of the rates of penetration at temperature (in degrees absolute) $T + 10$ to that at T is called Q_{10} then

$$\ln Q_{10} = \frac{10E_m}{RT(T+10)} \quad \text{so} \quad \exp(E_m/RT) = (Q_{10})^{T+10/10}$$

If we suppose that, besides the factors already mentioned, the rate of diffusion within the membrane depends upon the rate of activation of the particles, i.e. $\exp(-E_m/RT)$ then the product (Rate of diffusion) $\times \exp(E_m/RT)$ would be constant, so the expression $PM^{0.5}(Q_{10})^{T+10/10}/r$ should be constant irrespective of substance diffusing. This constancy is not, in fact, found. A difficulty in relating the permeability to the oil/water partition ratio is in the

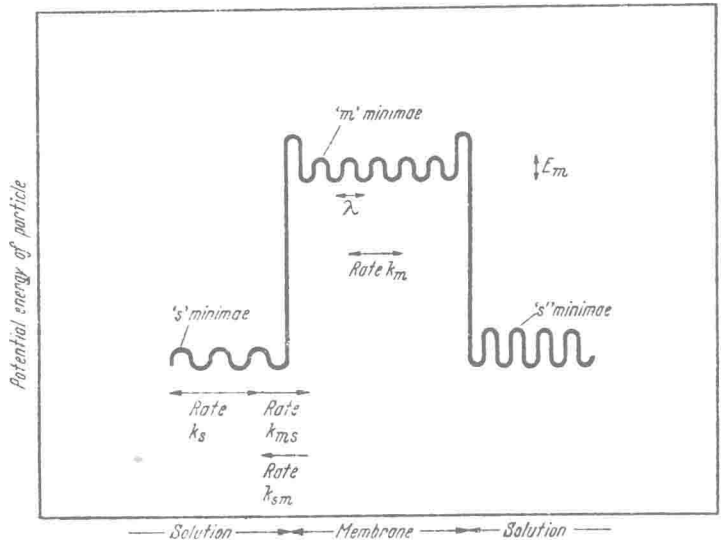

Figure 2. Diagram of potential energy of a particle in solution and membrane. There is no electrical asymmetry, the diagram applies therefore only to uncharged particles or to ions in the absence of a transmembrane potential difference.

choice of oil to simulate the lipid. COLLANDER (1947) has shown that the ratios vary considerably between different oils.

The potential energy diagram approach to the problem of permeability can be helpful; it has been extended recently by ZWOLINSKI, EYRING and REESE (1949). These authors calculated the free-energy changes for the whole permeation process, including the intra-membrane diffusion. The former comes to 11–15 Kcal/mol and the latter to 7–9 Kcal/mol for a number of polyhydroxy alcohols entering egg cells or *Chara*.

The permeability can be related to the potential energy diagram. The equation for P is given by:

$$P = \frac{k_s\lambda}{s + s' + 2k_s/k_{sm} + mk_sk_{ms}/k_{sm}k_m}$$

where k_{sm} is the rate constant for passage from solution to membrane, k_s is the rate in the solution, k_m is the rate in the membrane, k_{ms} is the rate from membrane to solution, s, s' and m are respectively the number of potential energy minimae in the two solutions and in the membrane, and λ is the spacing between the minimae in the membrane (see Figure 2).

(b) Penetration through pores

BOYLE and CONWAY (1941) discussed the selective permeability of the muscle membrane in terms of the relative sizes of the ions and the pores. Different hydrated ion radii will not only change the ease of entry into pores, but also will change the forces of adsorption between the ions and the membrane material as emphasized by LING (1952).

The sizes of the hydrated ions of Na and K differ considerably and are in opposite order to the sizes of the unhydrated ions. Figures for some hydrated ion diameters are collected in Table 2, part of which is drawn from B. E. CONWAY (1952).

Table 2

Ion	Hydrated ion diameter (Å)
K : Cl : Br : I : NO_3	3
HPO_4 : SO_4	4
Na : H_2PO_4 : HCO_3	4·5
Sr : Ba : S	5
Li : Ca : Cu	6
Mg	8
H	9

No quantitative relations have been established between the rate of penetration and the ionic size, but it is generally true that the larger ions (exceeding 4·5Å) pass membranes only with difficulty. It is to be recognized that mere slowness of penetration does not explain an ionic distribution differing from that predicted by physicochemical laws, it is necessary to invoke some difference in mechanism for the penetration, for example, a reduction in size caused by dehydration on one side.

When comparing the rates of penetration of various ions in a given sense through a membrane it is necessary to make allowance for the electrical forces acting; these depend upon membrane potential difference and ion valency. An elegant method, which

has been applied to frog skin and nerve, is to measure the transfer rate with the electrical field across the membrane held at zero so that there is no electrical force acting (see Chapters 6 and 10).

(c) Penetration by intermediate compound formation

When the rates of entry into cells of some substances of similar molecular weight are compared it is found that particular ones may cross the membrane much faster than others. This usually indicates that there is some special mechanism operative; in such cases it is usually possible to poison the special process, reducing thereby the rate of transfer to a value similar to that of other test substances. A good example is that of the entry of sugars into erythrocytes; the rates are high despite the large size of the sugar molecule, but the rates are reduced by Hg ions and many other poisons. That different mechanisms apply to the entry of various compounds is shown by such observations that narcotics reduce the rate of glycerol entry into erythrocytes, yet increase the rate of thiourea entry (JACOBS, 1950). The rate of glycerol entry depends on pH (WILBRANDT, 1941) and this also suggests intervention of a chemical process. JACOBS (1952) has classified mammalian red cells into three groups according to their behaviour to glycerol. Many species (dog, ox, sheep, cat) have cells which are penetrated equally readily by glycerol and similar non-electrolytes; the rate of penetration is unaffected by Cu ions. The erythrocytes of the ground hog and some rodents have a still higher permeability to non-electrolytes generally and are also insensitive to Cu. Finally, the cells of man and the rat are many times more easily penetrated by glycerol than by the similar compounds, and their high glycerol permeability is reduced reversibly by adding Cu ions (JACOBS, 1950). At the same time the temperature coefficient (Q_{10}) of the rate of permeation of glycerol is altered, that of the poisoned cells is about 3, compared with 1·3 before poisoning.

The conclusion drawn is that glycerol enters human and rat cells by a mechanism involving a part of the membrane which can be altered by Cu ions. In this respect it resembles the entry of sugars (p. 120).

The high permeability of red cells to the anions Cl and bicarbonate can be reduced drastically by treatment with low concentrations of tannic acid (JACOBS, STEWART and BUTLER, 1943). This does not alter the permeability to water and many non-electrolytes. Some other agents, e.g. butanol, increases the permeability to cations and diminish that to anions. Where there is this dual effect it suggests that there has been a change in sign of the fixed charges

in the membrane. The sign of the fixed charges determines which ions can most readily enter the membrane; cations are opposed by a membrane bearing positive charges, while anions enter it readily. Examples of the consequences of this effect will be met in the next section.

POTENTIALS AND ION PENETRATION OF SOME ARTIFICIAL MEMBRANES

The extensive work which has been carried out on artificial membranes makes it necessary to restrict discussion here to a few salient features. The references will provide material for further reading in this field. The aim of investigations with artificial membranes has been to provide model systems displaying the same sort of specificity as encountered in biological examples. Membranes possessing a high concentration of ionizable groups have been prepared either by incorporation of sulphonated polystyrene resin or protamine in collodion, or directly from various polymers. The acid polymers lose H ions to the surrounding solution so that the bulk of the membrane is negatively charged with respect to the aqueous phase, while use of basic materials like protamine gives rise to a positively charged membrane (ABRAMS and SOLLNER, 1953; SOLLNER, 1953; SOLLNER *et al.*, 1954; GOLDMAN, 1943; SCATCHARD, 1953; TEORELL, 1935 and 1951; MEYER and SIEVERS, 1936a and b). The acid membranes are cation exchangers, and permit passage of cations through their bulk much more readily than anions (which are kept out by electrical charge), and basic membranes are anion exchangers permitting readier passage of anions than cations. The net charge given to the solution by the ions derived from the membrane material means that a force acts on it when it is in an electrical field. This force causes the solution to move, and the direction of motion is determined by the sign of the charge and the direction of the field. This phenomenon is called electro-osmosis, it is further discussed in Chapter 2, p. 34. Here it will suffice to say that this cause of fluid movement can be brought into play by the natural potential difference between two unlike solutions, as well as by a field from an external source. When there is no obvious source of current, fluid movement due to electro-osmosis is often called anomalous osmosis, such movement is quite distinct from that taking place as the result of the ordinary osmosis (Chapter 2, p. 17). Through collodion membranes the water movement reaches nearly 1 ml cm^{-2} h^{-1} (ABRAMS and SOLLNER, 1953). If current is passed through a highly charged membrane (polymerized