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Membrane Transport

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IN discussing biological membranes one deals with a considerably higher level of organization than has characterized most of the material covered in the foregoing papers, even in the consideration of cellular biology, since one must deal with everything from the presumed limiting membranes of individual cells to entire layers of cells, each of considerable complexity and, in some cases, even consisting of several different cell types. However, in general, the problem is oversimplified here by disregarding whenever possible all details of structure which are not obviously concerned directly with the processes under immediate examination.

The animal body can be considered to be made up of a number of compartments separated one from another and from the external environment by a series of membranes. Each compartment has a more or less characteristic composition, often differing markedly from that of the adjacent compartment so that across these membranes there may be rather steep gradients of chemical concentration and often of electrical potential as well. The problem is to clarify the means by which various materials cross these membranes and by which the striking gradients are maintained. To some extent the penetration of various materials can be defined in terms of relatively simple forces; in other instances it is apparent that the movements of certain substances require the utilization of energy, a process which has been designated as active transport and which is currently the subject of numerous studies. In no case, however, can it be said that the mechanisms involved have been identified.

Consider, in a general fashion, the possible ways in which materials might cross biological membranes and the forces which might effect these movements. These considerations are based heavily upon the treatment of the subject of membrane transport as developed by Ussing. In general, the definable forces which produce movement across membranes can be classified as: (1) those due to gradients of chemical activity including differences in concentration or activity coefficients; (2) those due to gradients of electrical potential; and (3) those exerted upon solutes by the flow of solvent through solvent-filled channels. In addition, one finds in practically all biological systems movements of solute which are explained by none of these definable forces and which, therefore, are designated as "active transport." The definition of active transport is a rather arbitrary affair. As defined, it has the advantage of singling out those solutes which must be considered directly involved in the process by which metabolic energy is utilized to perform transport work. However, clearly all concentration or electrical gradients and flows in response to pressure or osmotic gradients ultimately depend upon metabolic work even though this may be remote from the particular membrane under consideration. So the requirement of metabolic work is not sufficiently exclusive for an adequate definition of active transport.

However, the definition does not include all processes which have specificities beyond simple diffusion and flow and does not include the process by which certain substances can cross membranes only, say, by combining with a specific carrier even though the movement is entirely downhill with respect to gradients of electrochemical potential. One must recognize, therefore, the arbitrariness of this definition. Presumably, when, and if, a better understanding of the discrete processes involved is attained, the need for the term active transport will disappear.

Of course, the manner in which solutes may cross membranes depends upon the nature of the membrane. If it is a continuous lipid layer, substances can pass this layer only by dissolving in it, although their passage through such a layer might be facilitated by combination with some component of the lipid layer which would enhance the lipid solubility of the solute. For such membranes, the permeability to particular solutes should, in general, be a function of lipid solubility. Indeed, observations going back many years have shown that in several chemical series the permeation of cells can be related to lipid solubility.[1] However, there are many features of cellular membranes which cannot be explained if they are assumed to consist of a solid lipid layer and it is generally believed that the lipid layer contains pores, through which water forms a continuous phase and through which the water soluble solutes pass. Indeed, it is only through such channels that forces produced by the flow of solvent can act. Since, as is pointed out later, there is evidence that solvent flow does produce movement of solute, it is apparent that such solvent-filled pores (and in this case, of course, the solvent referred to is water) are present in some membranes. For present purposes, it is convenient to accept the view that cell membranes generally consist of lipid layers penetrated by aqueous pores, the walls of which may bear an excess of fixed charged groups which will impede the passage of ionic species of the same sign. In some cases where membranes consist of more complicated structures than the

plasma membrane of single cells and may consist of an entire layer of cells attached to a common basement membrane, there may be reason to believe that the passage of water soluble substances occurs between the cells rather than through them. This appears to be the case in blood capillaries,[2] but probably not in others such as the mucosal lining of the alimentary tract and the skin of frogs and toads.

Perhaps the best-studied biological membrane system and the one in which theoretical considerations have been applied most explicitly is the frog skin which, in particular, Ussing has used as a model system for extensive studies.[3–5] Unfortunately, from the structural point of view, the frog skin is rather complicated, but perhaps it may be simplified for present purposes by making the probably valid assumption that only the basal layer of cells is involved in the transport processes of concern here. The skin can be stripped rather easily from the abdomen of the frog and stretched *in vitro* as a diaphragm between two chambers. It will then survive in this isolated state, perform its metabolic operations, and retain a well-maintained capacity to perform active transport for some hours. The sheet of tissue thus obtained consists of a loose layer of connective tissue, a continuous basement membrane, and several layers of epithelial cells. As one proceeds from the basement membrane toward the outer skin surface, the cells progressively become more flattened and keratinized and, presumably, lose their metabolic activities. In any case, it may be assumed for present purposes that all of the observed phenomena are attributable to the basal layer of cells—an assumption to some extent supported by the fact that toad bladder has been shown to exhibit many similar properties but to have only the single layer of cells corresponding to the basal layer of cells in the skin.[6]

Now, it has been known for many years that the frog skin generates an electrical potential, the solution in contact with the inner surface being positive with respect to the outside. It has also been known that the frog in the intact state is able to extract salt from its surroundings even though the outside concentration of salt is far lower than in the tissue fluids of the animal. For instance, Krogh[7] found that the frog which has a concentration of sodium in its extracellular fluid of something over 100 mEq/L (milliequivalents per liter) was able to take up salt from its surroundings when the outside concentration was as low as one hundredth of a milliequivalent per liter—a concentration ratio of 10000:1. Furthermore, the uptake was completely specific for sodium—no potassium or calcium was taken up even when the concentration was many times higher. One other unusual observation was made by Hevesy *et al.*[8] when the technique of using isotopic tracers was introduced. They found that the frog sitting in water had a net uptake of water several times greater than predicted from the measured influx of tagged water and the assumption that the flux in each direction across the skin should be proportional to the water activity in the solution of origin—in this case, the lymph or extracellular fluid of the frog and the water in which the frog was immersed. Observations such as this one led to the hypothesis that water was actively transported across such membranes (similar observations were made by Visscher and his associates using the intestine of the dog[9])—however, a simpler physical explanation is shown to make the assumption of active water transport unnecessary. One further observation on the intact frog might be mentioned before going on to the more detailed observations—this is the fact that the rate of uptake of water from the surroundings can be enhanced greatly by injection of the frog with posterior pituitary extracts.[10] Hence, the term amphibian water-balance principle was introduced to describe this activity, which, however, has been since shown to be at least qualitatively reproduced by the use of the purified polypeptide hormones of the posterior pituitary.

These, than, are the gross observations which Ussing and his collaborators set about to dissect by the application of isotopic tracers. Disregarding for the time being the net movement of water which in any case is negligible when the skin is bathed on both sides with solutions of equal osmotic pressure, Ussing showed that the forces acting on an ion to cause movement across the intervening membrane would produce a flux from one surface to the other described by the equation

$$M = -\frac{d\bar{a}}{dx}\frac{ART}{GNof}\exp-\frac{zF}{RT}\psi,$$

where M is the unidirectional flux across the membrane, $\bar{a}$ the chemical activity of the species under consideration, A the area of the membrane available for penetration by the species under consideration, G is a frictional coefficient describing the interaction between the membrane and a diffusing ion, No is Avogadro's number, z the valence of the ion, ψ the electrical potential, and f, F, R, and T have their usual meaning.[3] The equation contains several unknowns which are not determinable, and, therefore, it is not too useful in this form. However, if attention is directed to the ratio of fluxes from one side of the membrane to the other, the indeterminate quantities cancel out leaving upon integration the relatively simple relationship,

$$\frac{M_{1.2}}{M_{2.1}} = \frac{f_1C_1}{f_2C_2}\exp\left[\frac{zF}{RT}(\psi_1-\psi_2)\right],$$

which contains only experimentally determinable quantities, except perhaps the activity coefficients, which, however, also cancel out when the solutions on the two sides of the membranes are essentially the same.

The skin was set up between two chambers, each containing solutions vigorously stirred and oxygenated by a stream of air or oxygen. When the inside solution

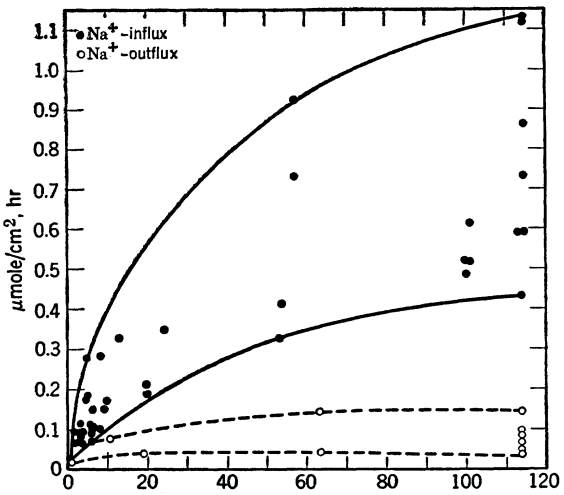

FIG. 1. Sodium fluxes across isolated frog skin as functions of outside sodium concentration [from H. H. Ussing, Cold Spring Harbor Symposia Quant. Biol. 13, 193 (1948)].

was Ringer's solution and the outside various dilutions of Ringer's, it was found[11] that the influx of sodium always was greater than the outflux so long as the outside concentration was not less than about 1 mM/l. The influx of sodium increased in nonlinear fashion as outside sodium was increased; there also was some small increase in outflux but this always remained small (Fig. 1.).

These, of course, represent merely some quantification of what was already well known—that sodium could be transported inward against combined chemical and electrical gradients and was, therefore, an active process. However, it was found that the chloride fluxes corresponded well to the predictions of the flux-ratio equation and that chloride movements could be considered entirely passive.[12] The sodium influx was generally greater than that of chloride so that there could be no question of bulk movements of elements of outside solution to the inside.[13] In general, the potential was higher when the sodium influx was greater and higher when chloride influx was decreased as could be achieved by treating the skin with 10^{-5} MCu^{++} solutions. It was proposed that the potential was produced by the transport of sodium and that the passive movements of other ions tended to reduce the potential.[14]

TABLE I. Unidirectional fluxes, net flux, and electric current in short-circuited frog skin [from H. H. Ussing, *The Relation between Active Ion Transport and Bioelectric Phenomena* (Instituto de Biofisica, Universidade do Brasil, Rio de Janeiro, 1955)].

	$\mu a/cm^2$			
	Na_{in}	Na_{out}	ΔNa	Current
I	20.1	2.4	17.7	17.8
II	11.1	1.5	9.6	9.9
III	40.1	0.89	39.2	38.6
IV	62.5	2.2	60.3	56.8
V	47.9	2.5	45.4	44.3

Studies of the short-circuited frog skin served to show that only sodium was actively transported and that, when the potential across the skin was kept at zero, there was a remarkable correspondence between the current flowing through the skin and the net movement of sodium ions[14] (Fig. 2 and Table I).

These observations, then, were compatible with the hypothesis that the only active process involved was the transport of sodium ions from outside to inside. No other ion, except—to a small extent—lithium could substitute for sodium,[4] and, in particular, potassium could not, although the complete absence of potassium from the bathing solutions very markedly reduced the transport of sodium—a circumstance to be mentioned again later.

These studies, then, have defined the behavior of the frog skin, but the question remains as to what kind of process might be involved in the transport of sodium

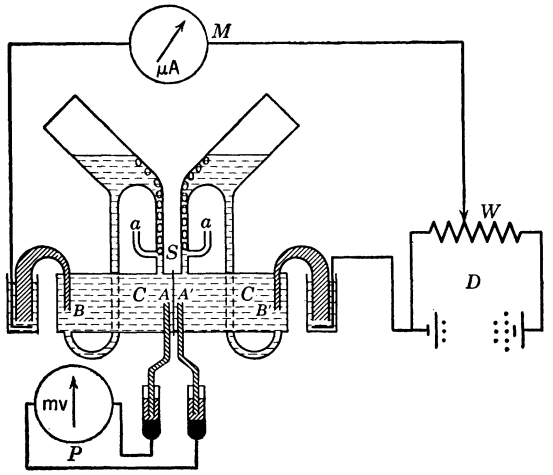

FIG. 2. Apparatus used for determining sodium fluxes and current in short-circuited frog skin [from H. H. Ussing and K. Zerahn, Acta Physiol. Scand. 23, 110 (1951)].

which, of course, is the major question in the field of active transport at present. Two additional types of study involving the amphibian skin have yielded information pertinent to this question.

One of the favorite theories of ion transport has involved the so-called redox or electron-transport pump, variations of which have been suggested by a number of investigators. These hypotheses are based on the assumption that oxidation and reduction of cytochrome iron are spatially separated and that hydrogen ions formed in the reductive step are available for exchange for some cation from outside the cell. The electrons then are passed along the chain, the cations taken up possibly moving with them, and the electrons finally are donated to oxygen to yield hydroxyl ions. Such a scheme has the stoichiometric limitation that only four electrons and four univalent ions can be transported per oxygen molecule consumed. By careful measurement of oxygen consumption and current flow (net

sodium transport) in the frog-skin system, both Zerahn[15] and Leaf and Renshaw[16] have been able to show that more than four ions are transported per oxygen consumed. The average figure obtained by Leaf and Renshaw (Fig. 3) was close to seven and some of the ratios were in excess of ten. Thus, even if all of the oxygen consumed were utilized in ion transport—a doubtful assumption—the ratio of transport to oxygen consumption is too great to be attributed to a redox pump mechanism. (Even higher ratios, going up to 20, can be obtained, if one divides the *change* in transport by the *increase* in oxygen consumption when sodium transport is increased in one of several ways.)

An alternative type of mechanism is suggested by other recent observations of Koefoed-Johnsen and Ussing.[5] The experiments were done with membranes in which maximum potentials were induced by reducing to a minimum the effective anion permeability. This

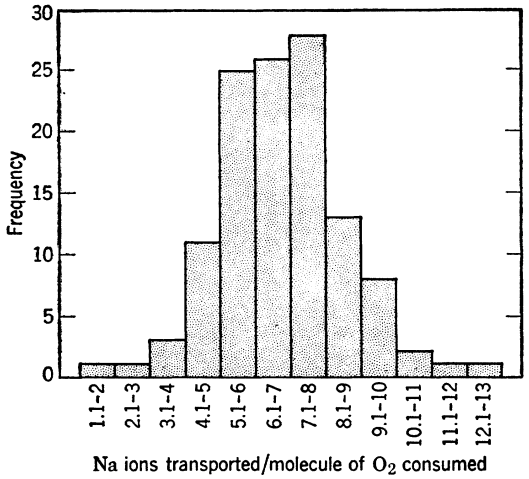

FIG. 3. Relationship of sodium transport to total oxygen consumption of isolated frog skin. Frequency distribution of 120 periods of simultaneous measurement [from A. Leaf and A. Renshaw, Biochem. J. **65**, 82 (1957)].

was done by treating the skin with Cu^{++}, or by replacing the chloride in the outside solution with sulfate to which the skin has a very low permeability. The concentrations of sodium and potassium on each side of the membrane were then varied systematically and the effect on the potential observed. Under these circumstances, the inside of the skin behaved very closely as if it were a potassium electrode, the potential across the skin decreasing approximately 60 mv with each tenfold increase in potassium concentration. Changing the potassium concentration on the outer surface of the skin had no effect, but changing the sodium concentration did, the potential increasing as sodium concentration was increased. This strongly suggested that the two surfaces of the cells making up the skin had quite different and specific properties—a fact that is apparent also from other properties. The two potentials thus behaved as diffusion or junction potentials at boundaries specifically permeable to single cations. The explanation offered is as follows: A sodium ion is extruded from the inner cell surface by forced exchange for potassium; that is, a charged carrier drops off a sodium ion and takes up a potassium ion. At the inner surface, by some exergonic reaction, the specificity of the carrier is changed and it gives up the potassium in exchange for another sodium ion. Thus, the sodium concentration inside the cell is reduced and the potassium concentration raised in a process which, in itself, produces no electrical potential. However, since the inner surface membrane is highly permeable to potassium, potassium diffuses out leading to a diffusion potential with the cell contents negative. The negativity of the cell contents tends to produce the uptake of a cation through the outer surface, but this surface being permeable only to sodium, it is a sodium ion which enters. The net effect is a cycling of potassium at the inner surface and a net movement of sodium ion from outer bathing solution to the inside of the skin. In the presence of chloride and a normal permeability to it, the potential is maintained at a lower level by the diffusion of chloride from outside to inside, and in the normal state the net movement of sodium and chloride, of course, must be very nearly equal.

This model is supported further by potential measurements made by Hoshiko and Engbaek[17] in Ussing's laboratory. By thrusting microelectrodes progressively through the skin, Hoshiko found the change to occur in two jumps which can be interpreted as representing changes occurring at the two surfaces of the active layer of cells.

This, then, provides a well-studied example of electrolyte transport by a membrane and it is generally hoped that, in principle, sodium transport is similar in other systems which are less accessible to isolation and study—such as the electrolyte transport in the renal tubules. Of course, here as elsewhere, very big questions remain—namely, what is the nature of the ion carrier? How does it derive its unusual specificity, and how is this specificity modified to provide the specific orientation of the transport process? Incidentally, mention should be made of the fact that the specificity for sodium on the outward trip (across the inner surface) in frog skin is not matched by a similar specificity for potassium on the inward trip, since varying the *p*H of the inside solution has an effect on the frog skin potential similar to that of changing potassium concentration,[11] suggesting the possibility that hydrogen and potassium may be interchangeable in this process. A similar situation appears to hold in the renal tubule.[18]

So much for the potential-generating and ion-transport properties of frog skin. There remains one aspect not mentioned yet—namely, the roles of diffusion and flow in the movement of water across the skin and the effect of such movements on the movement of solute. These phenomena again can be used to illustrate more-

general phenomena characteristic of biological membranes. Actually, even in the conditions already discussed, there is some net movement of water inward in an amount such as to make the net transport of salt result in no net change in solute concentration. However, this is so small as to be justifiably neglected. Under other conditions, however, there may be appreciable net movements of water. How may such water movements be brought about and what might be their effects on the fluxes of water and various solutes? Aside from the possibility of active water transport, a phenomenon existence of which is doubtful and which certainly is not required to explain anything in connection with frog skin, movements of water might be the result of hydrostatic or activity gradients. There are no appreciable hydrostatic gradients involved in the skin system so one is left with gradients of water activity or osmotic pressure. If one has only osmotic gradients, does this exclude the possibility of hydrodynamic flow through pores? It has been claimed that this is the case.[19] However, Ussing has illustrated with a simple model a mechanism by which hydrodynamic flow can result from a gradient of solute concentration only.[13] There are undoubtedly more-rigorous approaches,[20] but this is an easily visualized model. Consider a pore permeating a membrane impermeable to some solute contained in a solution on one side and not on the other. Consider the boundary of the pore at the end facing the solution. Just inside the pore, which the solute cannot enter, is a pure water solution of molar fraction one while just across the boundary of the pore is a solution containing a finite concentration of solute and having a molar fraction of water something less than one. Now clearly there will be a net diffusion of water from just inside the pore into the solution. But since the loss of water from the end of the pore cannot change its molar fraction, there is no reason why water should diffuse from deeper in the pore. Nevertheless, there will be a net movement of water through the pore. This then must be the result of a decrease in pressure at the end of the pore from which water has been lost and, hence, there actually is a hydrostatic gradient causing the water movement and, of course, hydrostatic gradients can cause flow through pores. It has been reported by Mauro that gradients of osmotic pressure across artificial membranes produce flow equal to that produced by the equivalent hydrostatic pressure.[21]

The effect of flow through a pore is to accelerate the diffusion of molecules in the direction of flow and to retard it in the opposite direction. Since the solvent drag force is inversely proportional to the diffusion coefficient, the effect in inducing asymmetry should be greatest for molecules as large as possible which can still pass readily through the pores. Andersen and Ussing have used thiourea and acetamide to examine the phenomenon.[22] Thiourea or acetamide were added to the solution on both sides of the skin (in this case, toad skin because it showed more marked responses to the addition of posterior pituitary extracts) in equal concentration, but the molecules in the outside solution were labelled differently from those in the inside solution. It thus was possible to measure the flux in each direction. Experiments were also done with labeled water. The rate of net movement of water was varied by diluting the outside solution. When the solution on both sides was Ringer's, there was very little net movement of water and the fluxes of the test substance were very nearly symmetrical (Table II). When the outside solution was diluted, there was a net movement of water inward and a marked asymmetry of the fluxes of thiourea and acetamide. When the log of the ratio of inward permeability constant to the outward permeability constant was plotted against the net water transfer, a linear relationship was obtained as predicted theoretically and the ratio of the slopes of 2.3 fits closely with predicted ratios of 2.4 and 2.7 for acetamide and thiourea, respectively (Fig. 4).

Of particular interest was the effect of adding neurohypophyseal hormone (Table II) which greatly increased the net water movements and the asymmetry of the fluxes, but had only a very small effect on the total water flux. Thus, it seemed to increase the area available for flow with very little change in the area available for diffusion. Such an effect might be the result of changing the shape of pores without changing their total area. In any case, these experiments seem to provide a clear indication of the existence of pores and of the production of bulk flow by osmotic gradients.

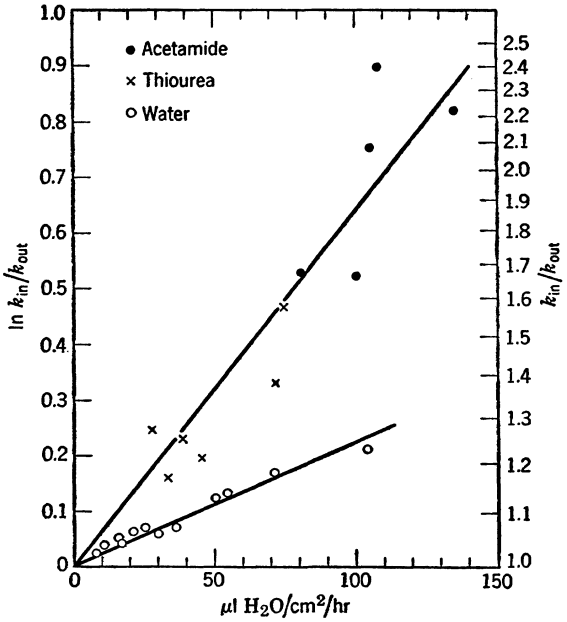

FIG. 4. Relationship between the rate of osmotically induced net water transfer (abscissa) and the logarithm of the ratio of inward to outward rate constants (ordinate) in isolated toad bladder [from B. Andersen and H. H. Ussing, Acta Physiol. Scand. 39, 228 (1957)].

TABLE II. Effect of neurohypophyseal hormone on permeability of isolated toad skin. Inside bathing solution Ringer's solution, outside solution as specified. K_{in}=inward permeability coefficient (cm/hr), K_{out}=outward permeability coefficient (cm/hr), and ΔW=net water transfer (μl/cm^2/hr), [from B. Andersen and H. H. Ussing, Acta Physiol. Scand. **39**, 228 (1957)].

			Before hormone				After hormone			
Date	Outside medium	Test substance	K_{in} ×10^4	K_{out} ×10^4	$\frac{K_{in}}{K_{out}}$	ΔW	K_{in} ×10^4	K_{out} ×10^4	$\frac{K_{in}}{K_{out}}$	ΔW
March 26	Ringer's	Thiourea	6.63	4.72	1.40	0				
June 11			10.5	8.08	1.30	0	278	278	1.00	0
June 14			11.5	9.75	1.18	0	106	109	0.97	2
June 22			11.5	13.7	0.84	3	247	223	1.11	12
June 23			12.4	14.1	0.88	2	64.7	62.3	1.04	1
June 25			3.17	4.47	0.71	1	7.71	8.45	0.91	1
					1.05	*			0.98	*
June 15		Acetamide					537	516	1.04	14
June 22							363	321	1.13	10
March 25	1/10	Thiourea	2.90	3.03	0.96	8	20.6	16.2	1.27	27
April 6	Ringer's		6.48	6.51	1.00	14	17.4	14.4	1.21	45
April 8			11.4	8.73	1.33	14	49.3	31.2	1.58	74
April 26			4.00	4.02	1.00	8	7.52	6.42	1.17	33
April 29			4.25	4.07	1.04	17	15.4	12.3	1.25	38
May 3			9.86	6.52	1.52	14	156	113	1.38	71
May 25		Acetamide					251	121	2.08	105
June 1							175	105	1.67	80
June 6							299	180	1.66	100
June 10							530	239	2.22	135
June 29			129	100	1.29	24				
April 6			81.5	76.1	1.16	16	196	81.5	2.40	108
April 26		Heavy water	3730	3510	1.06	22	5550	4510	1.23	104
May 3			4090	3890	1.05	20	4250	3750	1.13	50
May 9			3630	3420	1.06	21	4270	3730	1.14	54
May 16			4040	3760	1.07	28	4560	3850	1.18	71

They also make unnecessary any assumed active water transport by the amphibian skin. Similar phenomena also have been demonstrated to occur in some marine eggs,[23] and it is a reasonable conclusion that the earlier observation of unexplained high net water fluxes in intestinal loops has a similar basis.

The phenomenon of exchange diffusion is of interest with respect to the mechanism by which ions cross cell membranes. The concept was invented by Ussing[11] to rationalize the very high rate of isotopic exchange between the sodium in cells with that in their surroundings. Thus, with the potential inside negative relative to the outside and with the sodium concentration much higher outside, work is required to extrude sodium. If it is assumed that the inward movements are entirely by diffusion and that the outward movement proceeds at the expense of metabolic energy, then the energy requirements would exceed half of the total metabolic energy production of muscle in the resting state. Ussing proposed that sodium ions attached to a charged carrier might exchange for other sodium ions in the external solution or in the cell interior, the carrier making the round trip with sodium attached so that no net change in sodium concentration on either side of the membrane would occur. Yet, if the sodium on one side of the membrane were isotopically labeled, the process would result in movement of label.

The concept of exchange diffusion has been useful in the interpretation of a number of other phenomena some of which more directly are evidence for the idea of reversible combination of the same ion with a carrier so that exchange is greater than actual transport or movement of the free ion. For instance, Hogben[24] has studied the secretory membrane of the frog stomach using the Ussing technique. The stomach secretes hydrochloric acid, chloride against both electrical and concentration gradient, hydrogen ion with the electrical gradient which, however, is smaller than the very steep opposing concentration gradient. Sodium behaves passively. However, the striking phenomenon in connection with the phenomenon of exchange diffusion is the very large flux in the direction opposite to active transport (Table III). The flux from the secretory surface corresponding to the inside of the stomach, to the nutrient surface corresponding to the blood, is more than twice the membrane conductance as determined by the relationship between imposed current and developed potential. A considerable part of the chloride

TABLE III. Chloride fluxes in stomach mucosa of the bull frog [from A. Hogben, *Electrolytes in Biological Systems*, A. Shanes, editor (American Physiological Society, Washington, 1955), p. 176].

Net chloride transfer μ Eq cm^{-2} hr^{-1}		Net charge transfer μ Eq cm^{-2} hr^{-1}	
Flux N−S	10.65	Current	3.05
Flux S−N	6.38	H^+ secretion	1.20
	4.27		4.25

Mean conductivity: Start 3.21 m mhos
End 2.28 m mhos

TABLE IV. Na active transport and exchange diffusion in HK sheep red cells.

External cation	Na outflux	Difference
Na+K	3.5	2.7 E.D.[a]
Mg+K	0.78	
Mg+K+Stroph	0.16	0.62 A.T.[b]
Mg	0.16	

[a] E.D. = Exchange diffusion.
[b] A.T. = Active transport.

movement must occur in a form which does not contribute to the transfer of charge—presumably the same carrier complex by which the active transport is effected.

Another similar case involves the movement of chloride across the large intestine of the frog and has been studied by Cooperstein and Hogben.[25] In this case, there is no active transport, chloride fluxes being symmetrical when the electrical potential is zero. However, at various levels of potential, the flux ratio is lower than predicted from the electrochemical potential ratio. This could be explained if some part of the flux were owing to exchange diffusion and thus was not affected by the electrochemical potential gradient.

Finally, an example of quite a different sort involving sodium transport by the red blood cell. The red cell is rather unusual, in a number of respects, in that it is non-nucleated, lacks the machinery for oxidative metabolism, and, in general, transports sodium and potassium at rates several orders of magnitude lower than most body cells. Also, they contain a rather high chloride concentration, 60 to 70% of that in the plasma, whereas most other cells contain very little; since they have an extremely high permeability to chloride, with a half-turnover time, measured in milliseconds,[26] the presumption is that the distribution is passive and, therefore, that the electrical potential can be estimated from the chloride-distribution ratio. If this is true, the accumulation of potassium in this cell cannot be considered as a passive consequence of sodium extrusion, as is frequently considered the case in other cells. In any case, the slow rate of transfer and the ease of direct analysis make the red cell a suitable object for study and the relative simplicity of the red-cell metabolism somewhat facilitates the study of the metabolic correlates of ion transport.

Some data obtained with sheep red cells by Tosteson and Hoffman[27] in our laboratory illustrate the phenomenon of exchange diffusion (Table IV). When cells are incubated in normal plasma, an outflux of sodium of 3.5 mM/l/hr is observed. The removal of sodium and its replacement with magnesium very markedly depresses the outflux of sodium. Since the only type of process by which sodium leaves cells which we can visualize as requiring sodium on the *outside* is exchange diffusion, it appears that close to 80% of the sodium output from the sheep red cell is attributable to this process. When the potassium also is removed from the medium, sodium output is reduced even further to a level compatible with complete absence of active transport and which is the same low level reached when transport is inhibited with strophanthanthidin, a glycoside related to digitalis and a member of a group of powerful and apparently specific inhibitors of sodium transport. These results again suggest that the sodium extrusion is effected by a carrier-linked exchange for potassium. From the amount of glycoside required to inhibit potassium uptake by human red blood cells, Glynn[28] has estimated that there are less than 1000 transport sites on the surface of the human red blood cell, a disk 7 μ in diameter and perhaps 1 μ thick.

These similarities between membrane-transport phenomena in diverse systems and even in orders of vertebrates encourage one to believe that the processes involved fundamentally are limited in number and are not different in each cell type and species, and that the study of simpler systems may yield information applicable to more-complex and less-accessible ones.

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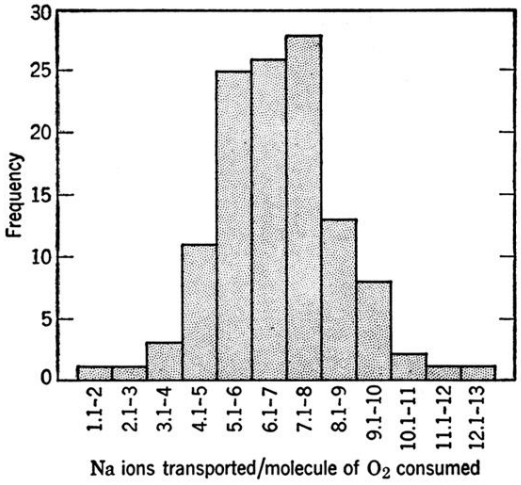

FIG. 3. Relationship of sodium transport to total oxygen consumption of isolated frog skin. Frequency distribution of 120 periods of simultaneous measurement [from A. Leaf and A. Renshaw, Biochem. J. **65**, 82 (1957)].