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EDITORS

Water and Electrolyte Metabolism

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INTRODUCTION

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The fact that so many of us are here this morning—well over 400 have enrolled —is an indication of how well-justified has been the enterprise of the three sponsoring Societies in organising this symposium. We, and the many others who cannot be present but will later read our "Proceedings" are grateful to them.

I am somewhat in a quandary as to the precise form my "Introduction" should take. I conceive the duty of the introducer to be threefold—to say something in general, but preferably sensible, terms about the subject of the symposium; to avoid stealing the thunder of the main contributors who are to follow; and to speak just so long that any late arrivals are in time for the first paper of the symposium proper, whilst those already present have not become impatient or bored.

Invariably the transition from one year to another, and more especially from one decade to another, is the signal for a flood of prophecies by people who tell us what we must expect during the time ahead. Among the crop which, last week, appeared in the British press and radio or television programmes was one which suggested that the focal point of scientific research would shift during the coming decade from nuclear physics to biology and in particular to the fundamental physical and chemical phenomena which constitute life. Such a move has, in fact, been discernible for some time, and the striking progress of organic chemists not only in unravelling the structure of protein and nucleic acids, but in showing how these latter substances control the reproducibility and the reproduction of the cellular matter, have already pointed the way.

But organic and organised cellular structure is not all; movement and change are also characteristics of living matter. As HOPKINS said, in an address to the British Association for the Advancement of Science as long ago as 1913: "Life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system". And again: "The cell constitutes a system which can maintain itself in dynamic equilibrium with its environment." Although HOPKINS was, at this time, emphasising the importance of specific catalysis in orderly sequence, he went out of his way to quote the dictum of PAUL EHRLICH: *"Corpora non agunt nisi liquida"*.

I stress the essential role of water because, in wonder at the magnificent achievements of the organic chemists, there is a perceptible tendency to ignore it or at least to take it for granted. We, here, know that water, along with particular inorganic ions in particular amounts, is absolutely necessary for the complex interactions of the organic substances which constitute living matter, but which without such interactions do not live.

Yet we do not know the precise functions of the ions; why they are required by

the cell in such definite quantities; how (or why) these essential ions are individually toxic and how by some mutual antagonism these toxicities may be cancelled out; how the cell maintains the correct ion concentrations (its "internal environment") in the face of a very different and variable external medium; or indeed many other things of fundamental importance.

It is right, and a necessary stimulus to further progress that we should from time to time take stock of our present knowledge and herein lies the value of our symposium today and tomorrow. During these discussions our speakers will consider water and electrolytes in relation to the diseased as well as to the healthy person, the pathological as well as the physiological factors. This too is right for both approaches must interest us whatever our professional label may be. The abnormal often yields evidence which elucidates the normal, and conversely the study of disease is inevitably and enormously facilitated by a knowledge of the processes occurring in health.

THE MOVEMENTS OF WATER AND SALTS THROUGH NATURAL MEMBRANES

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Today and tomorrow we shall be hearing about the movements of water and electrolytes in a number of different tissues, both normally and in disease. In this introductory lecture I thought it would be a good idea to look at water and electrolyte movements from the point of view of a single cell. To start with, it will be better not to name the cell, as once one calls it a nerve cell, or a muscle cell, or a red cell, a number of special features are introduced which complicate the picture.

Our generalised cell, then, will contain a solution of proteins and salts, and, at the pH that is likely to exist within the cell—say somewhere between 6 and 7 (HILL[1])—the protein will have a net negative charge. The cell will be bathed in a solution containing, probably, less protein—none at all if the cell is a unicellular animal living in the sea or a pond—and containing salts in different relative concentrations from those inside. If the cell membrane were freely permeable to water and salts, this pattern could not be maintained. The inequality of protein on the two sides of the membrane would lead to an osmotic inflow of water, and the salts would take up a Donnan distribution which would increase the osmotic flow still further and would be different from the observed distribution of ions. What I want to do is to talk about the features of the cell membrane that enable the characteristic pattern to be maintained.

Consider first the problem of water movements. The obvious question here, clearly, is: is there osmotic equilibrium across the membrane, or is cell volume maintained by the active pumping of water out of the cell against an osmotic gradient? In an amoeba, or other unicellular organism living in a pond, it is fairly clear that osmotic equilibrium cannot exist and the animal must, directly or indirectly, pump out water. In large multicellular animals the question whether the cells are in osmotic equilibrium is, rather surprisingly, still not quite settled, though it seems very likely that most of them are.

One way of testing whether osmotic equilibrium exists is to make a list of the solutes present on each side of the membrane, together with the concentrations in which they are found, and then to calculate the total number of osmotically active particles on the two sides and see whether they are the same. The difficulty with this method is that the list may be incomplete or, conversely, may include some solutes which are breakdown products of larger, and therefore osmotically less active, molecules. The method has been applied to red cells by HILL[2] in 1930, and more recently by DRABKIN[3], and to rat muscle by CONWAY AND HINGERTY[4].

A more direct method is to measure the freezing point depression of tissue homogenates. CONWAY AND MCCORMACK[5] froze tissues in liquid oxygen, then ground them to a powder, suspended the powder in isotonic saline, and measured the freezing point

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depression of the solution obtained. A complication of this method is that, with many tissues, the freezing point depression increases steadily with time—apparently as a result of autolysis (CONWAY AND GEOGHEGAN[6]), so that to find the original freezing point depression it is necessary to measure the rate of increase and extrapolate to zero time. CONWAY AND MCCORMACK measured the freezing point depression of homogenates of liver, kidney, diaphragm, and abdominal muscle, in both rat and guinea pig. Their experiments, together with those of BRODSKY *et al.*[7], and others, are reviewed critically by DICK[8], who comes to the somewhat cautious conclusion that "the assumption of osmotic equality does not seem to be unequivocally contradicted by any of the evidences so far available".

Yet another way of testing whether osmotic equilibrium exists is to measure the change in volume which occurs when cells are immersed in hypo- or hypertonic solutions. A difficulty here, apart of course from the practical difficulty of measuring the volume change, is that as water enters or leaves the cell the intracellular concentration changes, and therefore the extent to which the osmotic behaviour may be expected to depart from *ideal* also changes. If behaviour at infinite dilution according to the Boyle – Van 't Hoff law is given by

$$\pi V = nRT$$

then behaviour in more concentrated solutions can be described by

$$\pi V = \phi nRT$$

where ϕ is simply an arbitrary "osmotic coefficient" inserted to make the results fit. At infinite dilution ϕ tends to 1. Protein solutions tend to have high osmotic coefficients, probably because of the large size of the protein molecules compared with the molecules of solvent, and at high concentrations the osmotic coefficients change markedly with change in concentration. PONDER[9] has made a thorough study of the volume changes of red cells in solutions of different strengths, and finds that, though they swell in dilute solutions and shrink in concentrated solutions, both the swelling and the shrinking are less than would be predicted if the cells were ideal osmometers. But red cells contain about 30% of haemoglobin and, from the osmotic behaviour of haemoglobin *in vitro*, DICK AND LOWENSTEIN[10] have calculated the change in osmotic coefficient to be expected as the intracellular concentration changes, and claim that this change in coefficient can account for most of the deviation from ideal behaviour observed by PONDER.

There are, of course, various situations in the animal body where a cell membrane separates fluids of different tonicity. Not all secretions are isotonic with the plasma. The cells lining the salivary ducts, for example, cannot be isotonic with both plasma and saliva; similarly, the cells lining the bladder and ureters must, more often than not, have a tonicity different from that of the urine. But in this sort of situation no osmotic problem need arise provided that the surface of the cell presented to the lumen is impermeable to water. A more complicated situation must exist in the mammalian kidney where, when a hypertonic urine is being secreted, the net effect is that water is transferred from a stronger to a weaker solution against an osmotic gradient. At first sight this would seem, necessarily, to involve a water pump rather than a salt pump, but WIRZ, HARGITAY AND KUHN[11] have put forward an ingenious theory which not only gets round this difficulty but also makes sense of some otherwise puzzling features

of the anatomy of the kidney tubule. Dr. WIRZ's theory is discussed elsewhere in this volume (p. 100).

Turning now to the maintenance of the salt distribution, the problem is clearly a good deal more complicated. It is not possible to postulate that the high internal potassium and the low internal sodium are maintained by impermeability of the membrane to these ions: most cell membranes are not impermeable to sodium and potassium. Nor has it been possible to explain the ion distribution in terms of ion binding in the cell interior, although several attempts to do so have been made. There is very little doubt that small amounts of sodium and potassium do occur in a bound state inside certain cells: EDDY AND HINSHELWOOD[12] have demonstrated potassium binding in some bacteria; GOLD AND SOLOMON[13] gave evidence of a slowly exchanging sodium fraction in red cells; there are several pieces of evidence suggesting ion binding in mitochondria—in particular GAMBLE[14] has recently shown that rat liver mitochondria bind potassium in preference to sodium even when their membranes have been disrupted with the surface-active agent digitonin. Nevertheless, except in bacteria, it seems that the total quantity of ions bound represents only a small fraction of the amount present in the cell. In nerve and muscle fibres, the mobility and diffusion of sodium and potassium ions appear to be of the same order as in free solution. Mobility and diffusion can be measured by adding radioactive sodium or potassium intracellularly, either by injection or by immersing the tissue for a short period in a solution containing the labelled ion, and then observing the rate at which the radioactivity diffuses through the cytoplasm or moves under the influence of an applied electric field[15-17]. A possible criticism of these experiments is that the added ions might behave differently from ions already present, but this seems rather unlikely as most flux measurements show that intracellular sodium and potassium behave as single compartments[18-21]. Apparent deviation from single compartment behaviour in frog muscle[22-24] seems to be due to slight differences in diameter or permeability among the population of fibres, as HODGKIN AND HOROWICZ[25] find that single fibres do not show this deviation. It is anyway very difficult to explain the low sodium concentration on the ion-binding hypothesis unless a large fraction of the cell water is bound, which there is evidence against[2], and which would upset the osmotic equilibrium. There is other evidence against the ion-binding theory (see reviews [26-30]), but this will do for the present.

We are therefore left with only one alternative—that the distribution of sodium and potassium depends on the activity of the membrane, which must be able to move one or both of these ions against electrical or concentration gradients or a combination of the two. If the membrane pumped sodium out, the inside of the cell would be left negatively charged; this would lead to a gain of potassium and loss of chloride, and at equilibrium the Nernst relationship:

$$\frac{K_i}{K_o} = \frac{Cl_o}{Cl_i} = \exp EF/RT$$

should hold. In red cells the potential cannot be measured, but the potassium ratio is about 20 and the chloride ratio about 1.4, so the explanation will not work. It is necessary to postulate the pumping in of potassium as well as the pumping out of sodium, and this fits in with observations that depriving the cells of glucose or poisoning them with fluoride leads to a slowing of potassium influx as well as of sodium efflux[31-34]. In nerve and muscle the chloride ratio is difficult to determine, as it is

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not easy to get an accurate figure for internal chloride, but the potential can be measured. Under physiological conditions the potential is generally found to be slightly lower than would be expected from the potassium ratio[35–38] (but *cf.*[27]), but this in itself is not good evidence for the active accumulation of potassium, as the potential measurements may be slightly out, for example through junction potentials. In nerve, however, anoxia and metabolic inhibitors strongly inhibit potassium influx as well as sodium efflux, so here too it seems that both fluxes are active[16,21,39–41]. There is also evidence in nerve and in red cells that active sodium efflux and active potassium influx are linked together, since sodium efflux is reduced by lowering the external potassium concentration, and the effect cannot be explained by a change in membrane potential[16,34,42,43]. As similar behaviour is found in frog muscle[20,44–46], it seems likely that, in this tissue also, active extrusion of sodium is linked to an active uptake of potassium, though there are no published data showing the effects of metabolic inhibitors on potassium influx.

Whatever the mechanism that is responsible for the sodium–potassium exchange, it seems to occupy a very small fraction of the membrane surface. Some years ago cardiac glycosides—substances like digoxin and strophanthin—were shown to inhibit the active movements of sodium and potassium in red cells without affecting respiration or glycolysis[47]; detailed study of the effects of cardiac glycosides on the active and passive fluxes of sodium and potassium suggested that they acted on the pump mechanism[48]. At low concentrations their behaviour was such that it seemed reasonable to assume that a single molecule of glycoside could affect only one carrier site and, since appreciable inhibition could be obtained at concentrations so low that relatively few molecules were present, it was possible to get an estimate of the number of carrier sites on the cells. The estimate was of course an upper limit, as not every inhibitor molecule could be assumed to be sitting on a carrier site. From the effects of low concentrations of scillaren A, one of the squill glycosides, it was calculated that the number of sites on each cell responsible for the active influx of potassium could not be more than about one thousand[48]. This low figure does not demand an impossibly high turnover, but it does suggest that very little of the total area of the membrane is involved, as, though we have no very clear idea how big a site is, on any reasonable estimate a thousand of them will not cover much space.

In this connection, it is interesting that rather similar results have been obtained in experiments on the exchange of both chloride and glycerol across the red cell membrane. EDELBERG[49] showed that chloride entry could be appreciably slowed by quantities of tannic acid sufficient to cover less than 1% of the surface, and JACOBS AND CORSON[50] found that glycerol entry was markedly inhibited by minute amounts of copper ions. I think it is important to bear these results in mind when one comes to consider how the membrane might function in terms of its chemical constituents. Chemical analyses of the cell membrane, and the majority of the physical methods of investigation, give information about the structure of the membrane as a whole, but if only one very small bit of membrane is responsible for the permeability to a particular substance, the information may be irrelevant.

The active movements of sodium and potassium require energy, and this energy is provided by the metabolism of the cell. In nerve, muscle, the nucleated red cells of birds and reptiles and probably in most tissues, the energy comes from aerobic metabolism[6,41,51]. In mammalian red cells, which lack the cytochrome system, the energy

appears to come from glycolysis[31,32,52]. Since some cells use oxidative energy, and some energy from glycolysis, it seems a little unlikely that active transport is linked to any particular stage in the glycolytic or respiratory sequences; but the mechanisms in the two sorts of cell could conceivably be different, and a number of workers, in particular CONWAY[53,54], have sought to link active ion movements with the electron transfer associated with the cytochrome system. In favour of this "redox theory", CONWAY[27] and CAREY, CONWAY AND KERNAN[55] point out that active extrusion of sodium in frog muscle is inhibited by cyanide, which knocks out the cytochrome system, but not by dinitrophenol, which is thought to dissociate oxidation from ATP synthesis. Indeed CAREY, CONWAY AND KERNAN find a small increase in the rate of sodium extrusion when dinitrophenol is present. There is, however, some disagreement, as yet unexplained, between the effects of cyanide and dinitrophenol described by CONWAY and his co-workers and those found by KEYNES AND MAISEL[56] and by FRAZIER AND KEYNES[57]. Under conditions in which net sodium extrusion is taking place, FRAZIER AND KEYNES find that the ratio of sodium expelled to oxygen used is considerably greater than would be expected on the simple "redox theory." In nerve and nucleated red cells, which of course lack the large store of phosphagen present in muscle, both cyanide and dinitrophenol inhibit active movements.

If active transport is not linked to any of the individual steps in glycolysis or respiration, it seems reasonable to suppose that glycolysis and respiration are required merely as sources of ATP, and that it is the ATP that provides the energy for ion pumping. The same conclusion is suggested by the fact that dinitrophenol inhibits ion pumping in nerve and nucleated red cells, which depend on oxidative energy, but not in mammalian red cells, which depend on energy from glycolysis[16,33,51,52]. If ATP does provide the energy, then under conditions in which ATP synthesis is stopped, one might expect the decline of active movements to be related to the fall in ATP content. Such observations have been made in squid axons by CALDWELL[58,59], and in red cells by DUNHAM[60] and WHITTAM[61]. More clear cut evidence that ATP can provide the energy for ion transport, comes from experiments in which ATP has been added intracellularly to cells lacking any other energy supply. In squid axons poisoned with cyanide, CALDWELL AND KEYNES[62] found that sodium efflux was partially restored by injecting ATP or arginine phosphate. (Arginine phosphate and ATP are thought to be in equilibrium in the axon through the action of arginine phosphoryl transferase, just as in vertebrate muscle creatine phosphate and ATP are thought to be in equilibrium through the Lohmann reaction.) If the ATP was hydrolysed by boiling before being injected into the axon, efflux was not restored.

In red cells intracellular injection is not possible, but ATP may be made to enter the cells by making use of the "reversible haemolysis" described by SZÉKELY, MÁNYAI, AND STRAUB[63] in Hungary and by TEORELL[64] in the United States. If red cells are lysed by mixing them with a large volume of water, and then washed, the ghosts obtained are highly leaky; but if the cells are lysed in a relatively small volume of water, and tonicity is then restored by the addition of salt, the ghosts have relatively impermeable membranes and, in the presence of suitable substrates show active accumulation of potassium. In the absence of added substrates no accumulation occurs. GÁRDOS[65] showed that if the cells are lysed not in water but in an ice-cold 10% solution of the acid sodium salt of ATP, then after "reversal" they contain about 4 mg of ATP per ml and can accumulate potassium without any further addition of substrate.

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In view of all this evidence it seemed worth while to look at the ATP-ase activity associated with the cell membrane. Such ATP-ase activity has been described in squid axons by LIBET[66] and in red cell membranes by CLARKSON AND MAIZELS[67], HERBERT[68] and CAFFREY *et al.*[69].

During the past fifteen months, Dr. DUNHAM and I have been investigating the

TABLE I

THE EFFECT OF STROPHANTHIN ON THAT PART OF THE ATP-ASE ACTIVITY OF RED CELL GHOSTS THAT REQUIRES THE PRESENCE OF SODIUM AND POTASSIUM

Na concentration (*mM*)	*K concentration* (*mM*)	*Activity* (*mM P/l cells/h*)
0	0	0.75
		0.78
0	16	0.82
		0.84
16	0	0.73
		0.83
16	16	1.34
		1.37
In the presence of 10^{-4} strophanthin		
16	16	0.78
		0.85

Conditions of experiment: Duration 1 h, pH 7.0, temp. 37.2 °. Mg, concn. 0.5 m*M*; Cysteine 1 m*M*; ATP 1.5 m*M*; "Tris" was added to make the total cation concentration 162 m*M* in all samples. (Data from E. T. DUNHAM AND I. M. GLYNN, 1960—in preparation)

ATP-ase activity of red cell ghosts. The activity seems to be of two kinds, both activated by magnesium ions. One kind occurs in the absence of both sodium and potassium and, provided magnesium is present, is greatly activated by small amounts of calcium; when the ratio of calcium to magnesium is made too high, inhibition occurs. This part of the ATP-ase is unaffected by strophanthin, and there is no reason to connect it with active transport. The other kind requires the presence of both sodium and potassium and, for optimal activity, the ratio between them must be neither too high nor too

TABLE II

THE EFFECT OF POTASSIUM CONCENTRATION ON THE INHIBITION OF RED CELL ATP-ASE BY A LOW CONCENTRATION OF STROPHANTHIN

K concentration (*mM*)	*Total glycoside-sensitive activity* (*mM P/l cells/h*)	*Activity in the presence of 5×10^{-6} strophanthin* (*mM P/l cells/h*)	*Inhibition* (%)
1/4	0.188	0.027	86
1/2	0.364	0.134	63
1	0.619	0.290	53
2	0.778	0.498	36
4	0.855	0.686	20
8	0.926	0.782	15
16	1.02	0.823	19
32	1.04	0.964	7

Conditions of experiment: Duration 1 h, pH 7.2, temp. 37.2°. Na concentration 60 m*M*; Mg concentration 0.5 m*M*; "Tris" to make up 160 mmoles. 1 mmole of cysteine and 1.5 mmoles of ATP were present. At its highest, the glycoside-sensitive activity accounted for 50% of the total ATP-ase activity. (Data from E. T. DUNHAM AND I. M. GLYNN, 1960—in preparation)

low. This part of the ATP-ase is completely inhibited by strophanthin (Table I), as has also been reported by POST[70]. There is, furthermore, a remarkable resemblance between the effects of very low concentrations of cardiac glycosides on this fraction of the ATP-ase on the one hand, and on the active potassium influx in intact red cells on the other (*cf.* Fig. 1 and Table II). In both cases the percentage inhibition is high at low potassium concentrations, and decreases as the potassium concentration is raised, so that the overall effect is that the low concentration of glycoside appears to increase the Michaelis constant for potassium. One way of interpreting this result is to

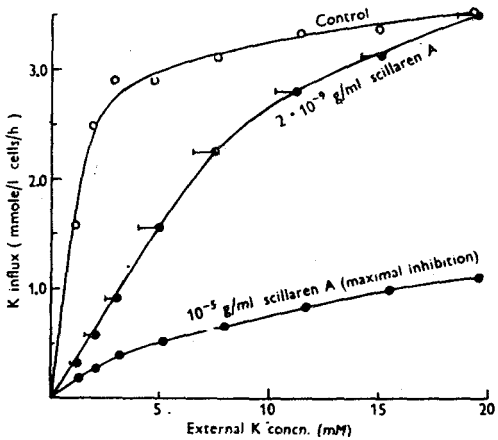

Fig. 1. The influence of potassium on the inhibitory effect of a very low concentration of scillaren A. Haematocrit *ca.* 3%. Cells were incubated with the test solutions for 3 h, ^{42}K was added, and the amount of ^{42}K which entered during the next hour was measured. The lowest curve represents maximal inhibition at each K concn. The short horizontal lines indicate the greatest error that could arise from the small increase in K concn. produced by the addition of ^{42}K. (From GLYNN[48]).

assume that the potassium ions and the glycoside molecules compete for the same site, but there are difficulties with this hypothesis.

Now consider two separate sets of properties of the red cell membrane. On the one hand we know from the experiments I mentioned earlier that the membrane can pump potassium in and sodium out, and that the two movements are linked so that one cannot occur without the other. The energy for these active movements certainly comes from glycolysis, and is probably made available as ATP. The active movements can be completely inhibited by cardiac glycosides, which do not interfere with ATP production, and at low concentrations of cardiac glycoside the inhibition can be reversed in a characteristic way by raising the potassium concentration. On the other hand, we have in the membrane an enzyme system which splits phosphate from ATP; which requires the presence of both sodium and potassium, neither alone being adequate; which can be inhibited by cardiac glycosides; and which, at low cardiac glycoside concentrations, shows the same reversal of inhibition as the potassium concentration is increased. One cannot help wondering whether the systems responsible for the two sets of properties are not the same—in other words: is the part of the ATP-ase that is sensitive to cardiac glycosides somehow responsible for the active exchange of sodium and potassium?

If this suggestion is right, then one would expect similar ATP-ase activity to be

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found in association with other cell membranes and, in particular, with nerve and muscle membranes. Unfortunately nothing is known of the effects of sodium and potassium on the squid axon preparation studied by LIBET, but in homogenates of crab nerve SKOU[71] has found two ATP-ases, one of which shows a very similar activation by sodium and potassium to that found by DUNHAM and me in the strophanthin-sensitive fraction of the red cell ATP-ase. Whether the nerve ATP-ase is similarly inhibited by cardiac glycosides, and, if it is, whether the inhibition can be reversed by potassium is not known. The dificulty in trying to find out the properties of ATP-ases associated with cell membranes is that it is only in exceptional cells—red cells and giant axons—that the membrane can be separated from the rest of the cell; and even in a giant axon whose axoplasm has been extruded, the membrane has a neurilemma adhering to the outside and probably still has mitochondria inside.

So far I have restricted my remarks entirely to the movements of ions into or out of cells. I want to finish by saying something about the movement of ions across cells. If cells are arranged to form a sheet, and the two faces of the cell have different transporting properties, then a net active movement from one side of the sheet to the other is possible. Such polarisation of cells, as it is sometimes called, may be reflected in the microscopic appearance—as, for example, in the brush border of cells of the proximal kidney tubule—or there may be no obvious visible difference between the two faces of the cell. Transfer of one sort of ion across a cellular membrane will lead to the setting up of an electrical potential which, if the permeability is right, will in turn lead to the transfer of ions of opposite charge. The net result will be that equal numbers of positive and negative ions are transported, but measurement of the electrical potential across the membrane may give a clue to which ion is actively moved. For example, in sheep the lining of the rumen possesses the power of taking up large quantities of sodium chloride. (The purpose of this absorption seems to be to regain salt secreted in the very copious saliva.) Though both sodium and chloride are absorbed, measurements of the potential across the rumen lining by DOBSON AND PHILLIPSON[72] show that it is only necessary to postulate active transport of sodium—the electrical gradient is sufficient to account for the chloride movement.

Another membrane that transports sodium chloride and that has been subjected to detailed study, in particular by Professor USSING and his associates in Copenhagen, is frog skin. Frogs, living in fresh water, tend to become depleted of salt, but possess the power of taking it up through the skin. Since the concentration of salt in the animal is much higher than it is in the pond, the absorbtion is clearly active; and since, when salt is being absorbed, there is a potential across the skin positive on the inside, it looks as though sodium transport is the primary event. That this is indeed so was shown by the elegant experiments of USSING AND ZERAHN[73] and of KOEFOED-JOHNSEN, USSING AND ZERAHN[74]. They put a piece of frog skin between two similar salt solutions, and arranged a counter-E.M.F. so that the potential across the skin was kept at zero. The current that had to be passed to hold the potential at zero was found to be exactly equivalent to the difference between the large flux of sodium from the outer to the inner face of the skin (measured with ^{24}Na) and the small flux of sodium in the opposite direction (measured with ^{22}Na). The net chloride movement through the skin at zero potential must have been zero. When the skin was treated with adrenaline, the relationship between current and net sodium flux broke down for a short time, apparently because chloride was secreted outwards, possibly from mucus glands.

Behaviour exactly like that of frog skin, but without the complicating effect of adrenaline, has recently been described in frog bladder by LEAF, ANDERSON AND PAGE[76]: sodium chloride is taken up from the urine and this uptake presumably helps to maintain body salt.

Animals living in sea water, or eating a very salty diet, are faced with the opposite problem—the problem of getting rid of excess salt. In fish this seems to be done by so-called "chloride secreting cells" in the gills, and in many marine birds there is a salt-secreting gland on the beak. It is said that the black-footed albatross can secret from its gland a 0.8–0.9 *M* salt solution at a rate of over half a millilitre a minute[76]. Whether the secretion of salt is primarily a secretion of sodium or of chloride is not known.

Recent work by KOEFOED-JOHNSEN AND USSING[77] suggests that the mechanism responsible for transporting sodium across frog skin may be the same as that responsible for the exchange of sodium and potassium across cell membranes. It has been known for some time[78] that for sodium transport to occur, it is necessary that potassium ions be present in the solution in contact with the inner face of the skin, even though potassium is not itself transported. KOEFOED-JOHNSEN AND USSING found that, in the absence of penetrating anions, the outer face of the skin behaves like an almost ideal sodium electrode whereas the inner face behaves like a potassium electrode. Direct measurements of the change in potential across the thickness of the skin by ENGBAEK AND HOSHIKO[79], using micro-electrodes, showed that the potential charge occurred in two jumps. Taking all these observations together, KOEFOED-JOHNSEN AND USSING suggest that the outer surface of the cells of the stratum germinativum allows passive movements of sodium but not of potassium; the inner surface allows passive movements of potassium but not of sodium; and that a pump at the inner surface pumps potassium into the cells in exchange for sodium. The system as a whole carries out sodium transport.

This is a very ingenious theory. It will be interesting to see whether a similar explanation will be found to account for salt absorption or secretion in the other tissues I have mentioned. It will be even more interesting if a more sophisticated version can account for the more elaborate events which occur in the kidney tubule. On the other hand, it is rather unlikely that sodium movements can always be explained in terms of sodium–potassium exchange. In the sea lettuce *Ulva lactuca*, SCOTT AND HAYWARD[80] find that sodium and potassium movements can be inhibited independently. In the gills of *Eriocheir*, KOCH[81] finds that sodium and potassium are pumped indiscriminately. And, of course, even where it is possible to explain salt transport in terms of a sodium–potassium exchange pump, this only brings us back to the problem: how does this pump work?

SUMMARY

The penetration of water and salts through cell membranes and membranes composed of sheets of cells is discussed. The way in which energy from metabolism is made available for the active transport of ions is considered, and some new work is reported suggesting that the active movement of sodium and potassium ions across the red cell membrane is intimately linked with the splitting of adenosine triphosphate by the membrane.

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DIRECT DETERMINATION OF THE BINDING OF ELECTROLYTES BY PLASMA PROTEINS

WITH SPECIAL REFERENCE TO Ca AND Mg

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Cations in the plasma exist in various physicochemical states; these are best known for calcium (Fig. 1). By ultrafiltration two fractions can be separated, the non-filterable one representing protein-bound calcium; with biological methods an ionized and a non-ionized fraction can be distinguished. This differentiation must also apply to the other cations.

The non-filterable fraction will be called the protein-bound fraction, whereas the filtrable fraction is indicated as the free fraction. In the case of calcium the latter contains not only the ionized fraction, but also a small quantity of complex-bound, but diffusible calcium.

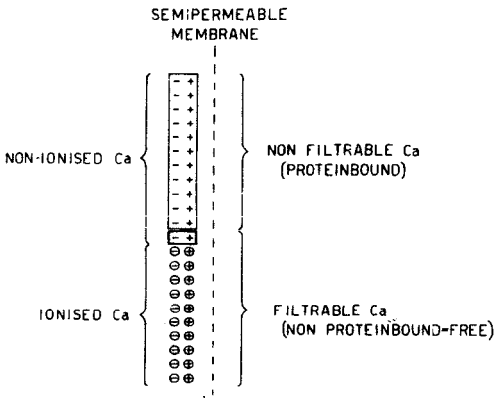

Fig. 1. Schematic representation of the various fractions of plasma Ca. Ca is indicated by a + sign, anions by a — sign.

In 1957, GERBRANDY[1] introduced a method for determining the protein-bound fraction of plasma constituents. It is based on the principle that a correlation must exist between the protein concentration and the concentration of any substance bound by it. GUTMAN AND GUTMAN[2] among others used this principle statistically to calculate protein-bound calcium from a large group of individual samples.

GERBRANDY, however, actively induced a rise in protein-concentration in the venous blood of the forearm by compressing the upper arm with a manometer cuff at a pressure of 90 mm Hg for a period of 7 min. Two blood samples were taken, one just before and one at the end of the compression period. By expressing the rise in concentration of any plasma constituent as a percentage of the increase in total protein