TRANSPORT OF IONS ACROSS CELLULAR MEMBRANES

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The ability to concentrate certain substances and to expel others seems to be present in all living cells. Indeed, with the possible exception of certain intracellular parasites, no organism could exist without being able to take up and excrete a number of substances so as to maintain the proper conditions for the processes connected with life.

Although most biologists probably have a rather clear idea of the meaning of ‘transport’ or ‘active transport,’ it may be useful to define the term in an unequivocal way. In accordance with Rosenberg (121), the term ‘transport’ or ‘active transport’ will be taken to mean a transfer of a substance against a chemical potential gradient. This definition is rather narrow; for instance, cases where a substance is found to pass faster along the concentration gradient than would be expected from simple physico-chemical considerations will not be included. The definition also excludes cases where a substance is concentrated in a cell by being bound to some cellular constituent as is the case with many dyes. As regards charged particles (ions) it is logical to restrict the term ‘active transport’ to cases where the transfer takes place from a lower to a higher electrochemical potential. The electrochemical potential difference for an ion species between two dilute solutions at the same pressure is equal to: \[ RT \ln \frac{c_1}{c_2} + RT \ln \frac{f_1}{f_2} + zF(\psi_1 - \psi_2) \] where \( c_1 \) and \( c_2 \) are the concentrations and \( f_1 \) and \( f_2 \) the activity coefficients of the ion in the two media, \( \psi_1 - \psi_2 \) is the electrical potential difference between them, \( z \) is the valency of the ion, \( R \) is the gas constant and \( T \) the absolute temperature. As is well known, the activity coefficient of single ions cannot be measured; but in dilute solutions it is permissible to assume that the activity of an ion is only determined by its charge and the ionic strength of the solution, and so it is possible to compute the activity coefficient.

The measurement of the potential difference across living membranes involves the use of liquid junctions, and if the solutions on the two sides of the membrane are not infinitely dilute or identical there will be an error due to the liquid junction potentials. If the liquid junctions are made through saturated KCl bridges, the junction potentials are reduced to a very small value. Nevertheless, this error makes liquid junctions undesirable in very precise physico-chemical work; but in most ordinary biological and chemical work the error due to liquid junctions is tolerated; as is well known, the standard methods of pH and redox potential measurements make use of liquid junctions.

The direct measurement of electrochemical potential differences in biological systems using electrodes which are reversible for the ion in question, is as a rule not feasible. If, for instance, one wishes to use silver chloride electrodes to measure the electrochemical potential difference for Cl, the solutions have to be saturated with silver chloride; but silver ions, even in very high dilution, are extremely poisonous to most living cells, so that, while avoiding a comparatively small error due to liquid junctions one destroys the system under observation.

This means that we shall only speak of active transport when work has to be done to transfer the ion across the membrane, whether this work is used to overcome a potential difference, a concentration difference or a combination of both. This definition has the advantage that, at least theoretically, a distinction is made...

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between transported and passive ions in cases of salt transport. Ions which cross a membrane due to electric attraction should not be regarded as being actively transported; the ions just move from a higher to a lower electrochemical potential although they may go from a lower to a higher concentration. Interest is thus focussed on the process of increasing the electrochemical potential of an ion at the expense of chemical energy derived from the metabolism. It may turn out later that the distinction between apparently transported and apparently passive ions is not justified. Nevertheless, the successful application in recent years of the Donnan principle on the distribution of ions between muscle and nerve cells and their surroundings (9, 132, 51) has given us hope that the multitude of different ion distributions in cells and organisms can be traced back to a finite number of more or less well defined transport processes involving a small number of ions.

Within the limits of a review article it would be impossible to discuss all recent papers with a bearing on active ion transport. In itself, however, our definition of active transport as a transport from lower to higher electrochemical potential suggests a limitation of the discussion to such systems where the definition can be applied. In other words, we shall have, primarily, to deal with biological systems where in the first place the concentrations of free ions on both sides of the membrane are known and where, secondly, the potential difference has been measured. Moreover we shall include cases where the direction of the electrochemical potential gradient can be estimated indirectly. These requirements, modest as they may appear, are only fulfilled in the case of a limited number of transporting organs, and mainly for monovalent inorganic ions. The transport of the divalent cations Ca++, Mg++ and Fe++ for example is hardly ripe for discussion, because these ions are largely present in complex form in the cells, the concentration of the free ions being low and mostly unknown. For similar reasons we cannot enter into a detailed discussion of the phosphate uptake in the cells. The concentration of free phosphate in the protoplasm is nearly always low relative to the concentration of phosphate esters, and there is always a risk of some labile phosphate ester being split during the extraction of the inorganic phosphate. Moreover phosphate may at least penetrate in three different forms namely as secondary and primary phosphate and as phosphoric acid. The subject of phosphate transport has quite recently been discussed by Sacks (126, 127) and Kamen and Spiegelman (65).

HYPOTHESES OF ACTIVE TRANSPORT OF IONS

Although the exact nature of the forces bringing about the transport of ions is not known, physico-chemical considerations show that, in many cases at least, the transport must involve chemical reactions between cell constituents and the transported ion. No other force possesses sufficient specificity to perform, for instance, the separation of Na and K ions from a mixture of these ions. This standpoint is clearly expressed by Spiegelman and Reiner (137). After having calculated the energy requirement for K accumulation and Na exclusion from a living cell they find that electric forces alone cannot account for the separation of K and Na whereas chemical reactions may easily supply the necessary energy. Recently Rosenberg (121) has given a theoretical treatment of the problem of active transport on a thermody-
He also excludes all forces but the chemical potentials as responsible for the transport processes. In the case of phosphate transport, for example, it is easy to imagine a formation of a phosphate ester at one boundary of a cell membrane and the splitting of the ester at another point in the cell. As a matter of fact there is some evidence that phosphate is actually taken up in muscle cells by being esterified with glucose.

The transport and especially the separation of Na and K has given rise to much speculation on account of the apparent similarity between these ions. This similarity should not be overemphasized, however. In their well-known model experiments with two aqueous phases separated by a layer of guaiacol, Osterhaut et al. (103) found an accumulation of K as compared with Na, evidently due to the higher affinity of guaiacol for K. On the other hand, according to Hodgkin (51), the tendency of Na to combine with a lipoid carrier might conceivably be greater than that of K because cations give rise to covalent linkages more readily when the atomic number is small (133).

If we accept the view that a chemical reaction is necessary between the ion to be transported and some cell constituent the general picture of the system must evidently be the following: 1) a carrier forming a relatively stable compound (complex) with the ion and 2) a system which can react with the complex so as to set free the ion, either by changing the carrier chemically or by supplying another ion which can replace the transported ion in the ion-carrier complex (78, 51, 150).

In order that a net transport be brought about it is necessary that the formation and splitting of the complex are spatially separated. A theoretical treatment of some hypothetical systems of this type is given in a very interesting paper by Franck and Mayer (35). Their basic assumption is that a substance a is transformed into b in one end of a cell (the word cell is taken to mean a compartment which may be a cell in the morphological sense of the word or it may be part of a cell, for instance the cell membrane) whereas in the other end of the cell a is again formed from b by some chemical reaction. This system is clearly well suited to bring about a transport of a. The authors point out, however, that if the diffusion coefficients of a and b are different, depending on the permeability of the cell walls, the system may perform active transport of a, b, the solvent or any combination of them. The calculations show that under favorable conditions such a system may act as a very efficient pump for both dissolved substances and for water. This must remind us that even a system capable of doing water transport would be able to carry salts from one place to another. Such transport would, however, lack the specificity of true ion transport. The system described by Franck and Mayer (35) may in short be designated a solute circuit system because the water transport results when a solute is circulated in the cell. It may be of interest to compare this system with the formally related fluid circuit system which Ingraham, Peters and Visscher (63) proposed for the absorption of fluid and salt in the intestine. In this system it is the fluid which is assumed to be circulated so that a more concentrated solution goes in one direction and a less concentrated one is returned, thus bringing about a net transport of salt. In the original form of this hypothesis the difficulty of explaining salt transport was merely replaced by the difficulty of explaining transport of fluid. Later, however,
Ingraham and Visscher (62) suggested that the pumping action might be produced by anomalous osmosis (136) brought about by an electric current through the pores of the epithelial membrane. To bring about the electric current some ion must be produced at one end of the pore and be removed at the other end. The origin of the transporting force is evidently the same as in the Franck and Mayer pump, but the anomalous osmosis hypothesis makes use of the electric rather than of the chemical component of the electrochemical potential difference. The formation of organic ions or NH₃ might conceivably produce the gradient required. Ingraham and Visscher (62) showed that NH₃ is indeed concentrated in the gut as compared with the blood. But the relation between NH₃ formed and salt absorbed is not a simple one and the authors do not consider it proven that the NH₃ has anything to do with the transport.

Although watery solutions and organic matter are normally considered impermeable to electrons, there is at least a remote possibility that systems may be set up in living cells which will conduct electrons over short distances (143). This possibility serves as a basis for a transport hypothesis which has been advanced to explain the formation of the cerebrospinal fluid (142) and the intraocular fluid (37). Stiehler and Flexner (142) conclude from a comparison of the concentrations of different substances in blood and cerebrospinal fluid that this fluid is formed by a secretory process in the chorioid plexus. It was observed that in this organ basic dyes move from the stroma to the epithelium whereas acid dyes move in the opposite direction. This was taken to indicate that the movement of ions (and water as well) was brought about by an electric current. The source of the electromotive force for this current was supposed to be the difference in oxidation-reduction potential between stroma and epithelium. Since the potential level (as measured by oxidation-reduction indicators) of the stroma is lower than that of the epithelium, the former might give up electrons to the latter. The electrons were assumed to pass from stroma to epithelium by means of reversible oxidation-reduction systems contained in the stromal-epithelial barrier. To maintain electro-neutrality cations must then move from stroma to epithelium or anions in the reverse direction. It is difficult to understand that the alleged electric current brings about a Na concentration in the cerebrospinal fluid which is higher than that of the plasma, whereas the K concentration is only 60 per cent of that of the plasma. Actually, as shown by Greenberg et al. (39) K penetrates more easily into the cerebrospinal fluid than does Na. Although the electron transfer proposed in the hypothesis may play a part, specific transport mechanisms are probably involved. The Stiehler and Flexner hypothesis implies that the difference in oxidation-reduction potential can be identified with the electric potential difference between the two tissues. This has never been proved for any tissue and a priori it is not very likely.

Lundegårdh (94) has proposed an explanation of the uptake of anions in plant roots which is closely related to the Stiehler-Flexner hypothesis. Lundegårdh points out that the change of valency of the Fe ion in the hemin group of a respiratory enzyme is well suited to effect an anion transport according to the scheme

$$\begin{align*}
\text{Fe}^{3+} + 3\text{A}^- & \leftrightarrow \text{Fe}^{2+} + 2\text{A}^- + \epsilon \\
\epsilon & \leftrightarrow \epsilon \\
\text{Fe}^{2+} & \leftrightarrow \text{Fe}^{3+} - \epsilon 
\end{align*}$$
the trivalent Fe attracting one more anion than does the bivalent Fe. The hypothesis now assumes a series of these Fe-enzyme molecules arranged across a boundary between two media of different redox potential and "owing to the wavelike proceeding oscillation of the Fe valency they will transport anions from the medium with higher oxidation power to the medium with lower oxidation power." Whereas, granted the freedom of movement of electrons from one Fe to the other, such a system may transport anions, it will at the same time transport hydrogen ions. Every electron arriving at the oxidation side means one positive charge neutralized which is practically identical with the disappearance of one H⁺. Similarly, the formation of the electron at the reduction side means the simultaneous formation of a hydrogen ion. Thus, what is transported is not simply anions as assumed in the hypothesis but anions plus hydrogen ions. Whether or not such a system will be suited to transport salts may ultimately depend on the mobilities of the different ions involved. A Teorell effect (147) due to back diffusion of H⁺ might conceivably result in the attraction of other cations. As a general explanation of transport processes these hypotheses share with other electric explanations the disadvantage of being unable to explain specific transport of one or few ion species. The specifically transported particles in the system just discussed are obviously the electron and the hydrogen ion.

DISCRIMINATION BETWEEN ACTIVE TRANSPORT AND DIFFUSION

Discussing the large differences in concentration of individual ions between the interior of living cells and the fluids surrounding them Krogh (80) writes: "... although permeability of the cell surface is of course a necessary corollary of the ion transport taking place, quantitative determinations of such permeabilities, in the general accepted sense of the term, can be made only by means of isotopes and even then require special precautions and conditions which are difficult to realize and verify, because the exchanges normally taking place are largely brought about by active transport." This statement rightly places much importance on the use of tracers in the study of the relation between transport and diffusion. As a matter of fact, whereas even the sensitive electrometric methods had failed to reveal any permeability to Na in most animal cells, the use of tracer Na has proven that the sodium ion can pass through practically all cell walls. But when one wants to evaluate this penetration in terms of active transport or diffusion he is up against considerable theoretical difficulty. The quantity which can be determined by adding a tracer at one side of a membrane and determining the rate at which tracer ions pass through may be designated the flux of the ion species in question (149, 150). The flux is defined as the amount of a substance which per unit time passes through unit area of the membrane in a given direction. If nothing else is known, however, a given ion flux may be the resultant of any combination of three processes, namely: a) free diffusion; b) exchange diffusion (see below); c) active transport. Although the present article should deal with point c only, a few remarks on the two types of diffusion might be justified because the active transport may be considered as the part of the flux which cannot be explained as diffusion. That cell walls generally are permeable to free ions is shown by the fact that they are conductors to a direct current, even though the resistance is very high as the excellent article by Cole (14) points out.
The theory of diffusion of ions through membranes and especially through living membranes is in a rather undeveloped state (23). Whatever the detailed mechanism is, it is evident that the flux in either direction must be the same when the ion in question is present at the same electro-chemical potential on both sides of the membrane. If the high K concentration in muscle and nerve cells as compared with the K concentration of the surroundings is due to a Donnan equilibrium then the K influx and the K outflux through the cell membranes must be the same and this flux value does not, of course, measure any active transport. If there is a difference in electro-chemical potential for any ion across a membrane, the influx and outflux will be different and the proportion between them will be a simple function of the difference between the electro-chemical potentials (see below).

The term exchange diffusion has been introduced to cover the phenomenon (83, 148) that an ion species crosses a membrane by combining with some carrier molecule which is part of the membrane or which cannot leave the membrane due to a selective solubility in the membrane phase. Due to the thermal movements the carrier-ion complex may alternately come into contact with either solution. When in contact with one of the solutions the complex may exchange the bound ion against one from that solution; but if the carrier-ion affinity is high, the carrier will always be saturated with the ion in question and the same number of ions will be carried from left to right as from right to left. The flux of the ion, as determined by the tracer method, would be nearly the same in both directions even if there were a considerable difference in electro-chemical potential for the ion across the membrane. The exchange diffusion thus simulates active transport and, as a matter of fact, both processes are mediated through carrier systems. They differ in that exchange diffusion does not require work and that it cannot bring about a net transport. On the contrary, there will always be some leakage through the exchange diffusion system because the affinity between ion and carrier is not infinite. The rôle of exchange diffusion for the exchange of substances across living membranes is at present unknown. We know that different molecules, having approximately the same size permeate the cell sometimes at very different rates. According to Reiner (119) this may indicate that many substances flow, not as free molecules but as adsorption compounds: solute—colloid. Reiner also gives a mathematical treatment of diffusion in such systems. The structure suggested for the cell membrane by Lundegårdh (88) would evidently lead to exchange diffusion. He assumes the membrane to be a Langmuir monolayer containing acid and basic groups which sometimes turn so that they are alternately in contact with both surfaces.

Brooks (10) believed that the rapid passing in and out of cations as well as anions in Nitella should be regarded as passive exchange. But his explanation differs somewhat from that given above. He assumed that the cell membrane possesses a mosaic structure so that some parts of the membrane were only permeable to anions and others only to cations. Such a membrane would, however, allow a leakage of positive and negative ions simultaneously and the system would hardly give any exchange-diffusion effect. Even though the explanation given by Brooks is not entirely correct the exchange in Nitella might well be due, in part at least, to exchange diffusion. The very possibility of this process at present limits the conclusions which
can be drawn from tracer experiments concerning the rate of active transport and of true diffusion. Used with proper care, however, the isotopes have provided a most valuable tool for the study of transport processes.

ACTIVE TRANSPORT OF CATIONS

*Does a K-transport Take Place in Animal Cells?* Whereas a simple analysis may show that the ionic composition of a cell is likely to be the result of active transport, it is far from easy to determine whether or not any specific ion species is being transported. A good example is found in the current discussion on the reasons for the high K content in most living cells. It is well known for instance that muscle cells are permeable to K ions. Most workers seem to be of the opinion that the greater part of the K in the cells is free (32, 33, 9, 80). This again implies that the K must either be transported actively into the cells or it must be held in equilibrium with the outside K due to electric attraction. In recent years the view has gained much ground that the cellular K not only of muscle but of animal cells in general stands in Donnan equilibrium with the outside K. The work of Conway and collaborators (9, 15, 16) has contributed much to our understanding of the ion distribution between cells and surroundings. In its original form the theory of Boyle and Conway (9) explained the distribution of K and Cl as the result of a double Donnan equilibrium, the system containing indiffusible anions (phosphate esters and proteins) inside the membrane and an indiffusible cation, Na, at a high concentration outside; K, H, Cl and a number of other small ions were free to diffuse in and out. The experiments of Fenn (33), Steinbach (140) and Heppel (45) show, however, that the muscle fiber membrane is permeable to Na. Indeed, in experiments with rats, having an increased Na content in the muscles as the result of feeding a low K diet to the animals, Heppel found that injected Na would equilibrate with the cellular Na within an hour, indicating a rapid Na exchange across the fiber membrane. Dean (27) therefore proposed to modify the Boyle-Conway theory by introducing the concept of a continuous extrusion of Na from the fibers by some pumping devise. It is obvious that the distribution of passive ions would be the same whether the difference in Na concentration between cells and surroundings were maintained due to Na impermeability or due to a constantly working active transport. Krogh (80) also strongly advocated a rapid active elimination of Na from the fibers, a view based on calculations indicating that Na entered the fibers faster than K did. Conway (15) calculated the minimum energy required for the extrusion of sodium from the normal frog sartorius if sodium entered as fast as potassium. The result was that more than the energy available from the metabolism of the resting muscle would be needed, so that the permeability figures for Na must be too high. Nevertheless Conway now admits that an active extrusion of Na from the fibers must take place (16). The concept of exchange diffusion, in fact, bridges the gap between the views of Krogh and Conway because the part of the Na influx into the fiber which is due to exchange diffusion does not require active extrusion. Boyle and Conway (9) based their theory on experiments with isolated frog sartorii and although there was a very good agreement between theory and experiments at outside K concentrations exceeding about 10 mEq/l, the P.D. measured at physiological
K concentrations fell too low (at 5 mEqv. K/l the P.D. was 59.4 mV. against the calculated 78 mV.). This apparently means that the process responsible for the P.D. (Na extrusion from and formation of phosphate esters in the cells) does not suffice to keep back the K in the cells when the proportion between inside and outside K becomes too high. Graham and Gerard (38) using very fine capillary electrodes have measured the resting potential of single muscle fibers from frog sartorii. The values with normal K concentration in the outside solution average 62 mV. with some values as high as 80 mV. Although the high values would correspond to a Donnan equilibrium for K, most values are lower than corresponding to an equilibrium. This is, however, what could be expected if the fiber is slowly letting Na leak in while K is leaking out. It should be remembered that isolated muscles and fibers are not normal. It is therefore highly significant that Wilde (154, 155) has been able to verify Boyle and Conway’s predictions in experiments on live rats. A stumbling block for the acceptance of Boyle and Conway’s theory has been that it demands a Cl concentration of the fiber water of about the same magnitude as the K concentration of the blood plasma. The striated muscle fibers, however, were considered free from chloride (33). But recently Heilbrun and Hoagland (41) found that the standard methods for Cl analysis on muscle gave systematically low figures. Wilde (154) confirmed this and worked out a more reliable Cl determination. Using this method he succeeded in demonstrating a considerable difference between the Cl space of rat muscle on the one hand and the inulin and saccharose spaces on the other hand, indicating that part of the Cl had to be in the fiber water. Moreover there turned out to be a significant correlation between the concentrations of fiber chloride and plasma potassium. In order that the equilibrium condition \( (K_i) (Cl_i) = (K_o) (Cl_o) \) be fulfilled it was necessary, however, to assume the activity coefficient of fiber K to be only 0.55 indicating that part of the K in the fibers is bound. There is indeed reason to believe that part of the muscle K is bound more or less firmly. Some evidence was presented by Mullins (100) and Szent Gyorgyi (144) that the principal protein of the muscles, myosine, binds potassium specifically. Brues, Wesson and Cohn (11) following the exchange of K in cultures of embryonal muscle, found that the uptake curve for isotopic K could best be explained as the sum of three independent exchange rates which they tentatively considered to be: 1) exchange of interspace K, 2) exchange of free K in the fiber and 3) exchange of bound K in the fiber. Of course these findings might be explained differently. The same type of uptake curve would be achieved for instance if the fibers consisted of two phases with different permeability; yet the assumption that some of the K is bound might well be correct.

It thus appears that two factors contribute to bring about the high K concentration of the muscle fiber, namely the Donnan equilibrium and to a lesser degree the specific affinity between K and myosin. There is indeed little reason for assuming any active transport of K into the fibers.

A Donnan distribution of K\(^+\), Cl\(^-\), HCO\(_3\)\(^-\), H\(^+\), OH\(^-\) and other small ions seems to be realized in many animal tissues other than muscle. Shanes (132) working with spider crab nerves, found them to be freely permeable to KCl, but having a very low permeability to Na. At increased outside K concentrations the P.D. and the
ion distribution corresponded to the type of Donnan equilibrium described by Con-
way et al. for muscle. At low K concentrations, just as was the case with frog sartorii, there was a marked divergence between calculated and found P.D. values.

Hodgkin (51) found that small changes in external K concentration caused large and rapidly reversible changes in membrane conductance in the squid nerve fiber. Rb and Cs ions had a similar effect. Na and Li had to be added in 40 times higher molar concentration than K to bring about a similar effect. This must mean that the fiber membrane is much more permeable to free K ions than to free Na ions. (The low rate of Na penetration relative to the K penetration is also clearly seen in the tracer experiments performed by Rothenberg and Feld (123)). The fact that the outside concentration of K has such large effect on the membrane conductance was now used in an ingenious way to demonstrate that K is given off from nerves during activity and taken up again during recovery (52). During the experiments the nerve fibers were submerged in oil; the outside water phase was thus reduced to a thin film where the minute amounts of K leaving or entering the fiber could bring about measurable concentration changes. The reabsorption of K by the nerve fiber is probably of electrostatic nature, however, possibly as a result of the active Na extrusion.

At present there is little reason to assume any active K transport in nerve. This view is very clearly expressed by Hodgkin (l.c.): "It makes little difference to the potassium distribution whether Na is kept out by an active process or by a semi-
permeable membrane. In either case the ratio of activity of potassium inside the membrane to that outside should be given by the expression $e^{EF/kT}$. The activity ratio could only exceed the value defined by the resting potential if active transfer of K took place. At present there is no clear evidence to show that the activity ratio does exceed this ratio, so that neglect of active transfer seems reasonable in a working hypothesis."

Although the necessary P.D. values are not available for other cell types with high K and low Cl concentration it seems at present reasonable to ascribe the ionic com-
position of these cells to a Donnan equilibrium of the type found in muscle and nerve. Indeed, clear-cut examples of true active transport of K in higher animals are few, up to the present. There is reason to believe that K can be absorbed in the gut of fishes to a lower concentration than that of the plasma (135). Likewise, a true K transport may perhaps take place in the kidney tubules. On the other hand Krogh was unable to find any K uptake in a number of fresh water animals (frog, goldfish and others) which take up Na eagerly even from exceedingly dilute solutions (79).

Certain crabs possess the ability to take up K as well as Na from the surrounding water. This has been demonstrated for the wool-handed crab, Eriocir sinensis (79) and the common shore crab, Carcinus maenas (129). This cation uptake which does not discriminate between Na and K calls for further study. Especially, it would be interesting to know the direction and magnitude of the electric potential difference across the gill epithelium of these animals during active ion uptake. Until such determinations are available it is impossible to decide whether a true transport of K (and Na) is going on or whether the salt transport is really an anion transport.
Potassium Transport in Plant Cells. Although K as well as many other cations are accumulated in the protoplasm and the cell sap of most plants, it is by no means certain that the accumulation is generally due to active K transport. In Nitella and Halicyclis the cell sap is negative relative to the outside solution under conditions similar to those prevailing during the uptake process. The material at hand does not make it clear, however, whether the P.D. always suffices to bring about the K accumulation. In root tips there is likewise a potential difference between the outside solution and the cut end of the root, which as to sign and magnitude would explain the transfer of cations from the medium to the bleeding sap (Lundegårdh (91)). It is important to note that the contact at the cut end of the root tip is made with the bleeding sap and not with the cell sap of the epidermal cells. Therefore we cannot tell whether the cation accumulation in the cell sap is of passive or active nature. On the other hand there is often a close correlation between the cation uptake of the whole root tip and the P.D. (130). The cation uptake in root cells will therefore tentatively be discussed under the heading anion transport.

There are, however, certain plant cells where a more or less specific K transport can be demonstrated. Pulver and Verzar (114, 115) found that yeast cells would take up K from the medium during fermentation and give up this K again at the end of the fermentation. This K uptake has been studied more closely by Conway and O’Malley (19, 20, 10) and by Rothstein and Enns (122). It turned out that the K uptake consisted in an exchange of hydrogen ions against potassium ions. If the yeast cells were allowed to fermentate in an unbuffered solution containing KCl they would take up K and give up H until the pH of the medium reached about 6.6. If a similar experiment was made with NaCl in the medium instead of KCl, hardly any Na was taken up by the yeast; and although a H+ excretion was observed the pH of the medium was only lowered from 4 to about 3. In neither case was any Cl taken up by the yeast. At first Conway and O’Malley assumed the peculiar K-H exchange to be due to a Donnan equilibrium in analogy with conditions in animal cells. Later, however, they altered this view because the pH of the content of the yeast cells was found to be much higher (5.8-6.2 in fermentating yeast) than required by the Donnan equilibrium. The authors therefore offered the explanation that the cell content consisted of two regions of which the outer compartment, having a very acid reaction, was in Donnan equilibrium with the outside, whereas the rest of the cell was separated from the outer compartment by a special cation impermeable membrane. Such a system would lead to K accumulation only in the outer compartment. As this would hardly account for all cellular K, the authors suggest a mechanism actively transfering one of the ions, K or Na, across the boundary between the acidic region and the rest of the cell. As yet, the region with high acidity has not been demonstrated directly. Whereas the process bringing about the forced exchange of H against K is entirely unknown, Conway and Brady (17) have been able to demonstrate that the H ions excreted originate from succinic acid and acid-labile carbon dioxide. It was found that with one part of yeast to 0.6 parts of a

\[ A \text{ similar K uptake is also found in certain bacteria (84) and possibly in other micro-organisms (131).} \]
5 per cent pure glucose solution, up to 0.2 per cent succinic acid was found in the suspending fluid. With KC1 in the suspending fluid the amount of succinic acid found in the medium was much lower, but there was a corresponding accumulation of succinate along with the K in the cells. However, the succinic acid formed did not account for all the H given off, the remaining part being derived from carbonic acid.

Another spectacular case of K transport is found in the much studied marine alga *Valonia macrophysa*. Here the cell sap is positive (about 8 mV) relative to the sea water and despite this the K concentration of the cell sap is about 500 mEq/l against 12 mEq/l in the surrounding water. The Cl concentration of the sap is also higher than that of the surrounding medium (597 mEq/l against 580), but due to the P.D. the Cl might well be concentrated passively.

The rate of KC1 transport into the sap of *Valonia* rises with increasing pH. This fact speaks in favor of Osterhout's assumption that the K crosses the cell membrane (and the vacuolar membrane) as a complex with some organic anion; a high pH favours the complex formation. A true active transport of K is obviously at work in *Valonia*. This view is expressed by Blinks (7) as follows: "Thus it appears to be the K ion which, if not responsible for the total P. D. of *Valonia*, nevertheless serves as the electrical indicator of various processes which are probably intimately connected with the accumulation of this element." The phosphate metabolism of *Valonia* seems to be very closely related to the K uptake. Thus Mullins (101) has shown that the rate of K accumulation increases when the external phosphate concentration is raised.

**Active Na Transport.** It has already been mentioned that an active extrusion of Na seems to be at work in most animal cells. In nearly all cells the Na concentration is lower than that of the tissue fluid. The use of tracers has given convincing evidence that Na can pass into nearly all cell types (45, 123, 95). Therefore, although it may be convenient, in electrophysiological considerations for example, to regard the cells as Na impermeable, this is not absolutely correct and the explanation of the function of the cell is incomplete if the effect of the Na transport is not taken into account.

For the following discussion of the Na transport it will be convenient to distinguish between cells where the transport serves mainly to maintain a more or less constant composition of the cell and such cells where a net transport takes place across the cell. The transport mechanism might well be the same in both cases; but the difficulties facing the experimenter are much greater in the first case.

The availability of isotopic tracers made it possible to determine the 'flux' of nearly all ion species across the cell membrane. In the case of muscle and nerve the part of the flux which is free diffusion can be shown to be very small indeed. Assuming the K ions to be distributed according to a Donnan equilibrium it can be shown (16, 83) that

\[
\frac{M_{\text{Na(out)}}}{M_{\text{Na(in)}}} = \frac{c_{\text{Na}(l)} \cdot c_{\text{K}(o)}}{c_{\text{Na}(o)} \cdot c_{\text{K}(l)}}, \text{ where } M_{\text{Na(out)}}
\]

and \(M_{\text{Na(in)}}\) denote the diffusion flux of Na outwards and inwards, respectively, and \(c_{\text{Na}(l)}, c_{\text{Na}(o)}, c_{\text{K}(l)} \) and \(c_{\text{K}(o)}\) denote the Na and K concentrations. The activity
coefficient for the monovalent ions is assumed to be the same within the fiber and outside; but even gross deviations from equal activity coefficient in the two phases cannot alter the main conclusion that the out diffusion does not contribute significantly to the total Na outflux ($c_{Na(i)}$) is much smaller than $c_{Na(o)}$ and $c_{K(o)}$ is much smaller than $c_{K(i)}$; $M_{Na(out)}$ is therefore much smaller than $M_{Na(in)}$; the total influx and outflux, however, are the same). But it still remains to be decided what part if any is played by exchange diffusion before we know the rate of active Na transport. Krogh's hypothesis (80) that the Na outflux is identical with the transport is naturally very attractive; but in the case of muscle, Conway's calculations (15) indicate that the Na extrusion must be slower than indicated by the exchange of radio sodium. The striated muscle presents unusually good material for deciding whether it is energetically possible to identify the Na flux with the transport, because the resting metabolism is low and the Na exchange high. This problem was the subject of the preliminary study by Levi and Ussing (83). The rate of Na exchange in the fibers was determined as follows: The isolated muscles (frogs' sartorii) were equilibrated with Ringer, containing Na$^{24}$ for about two hours. The process of washing out the Na$^{24}$ was then followed over a period of several hours. The logarithms of the counts of Na$^{24}$, washed out in equally long periods, when plotted against time, lie on a curve which can be resolved in two straight lines. The slopes of these lines are taken to indicate the rates of Na exchange between washing fluid and interspaces, and between interspaces and fibers, respectively. If this interpretation of the result is correct, the half renewal time of interspace Na is about two minutes whereas the half-renewal time for the fiber Na is about 30 minutes. Under the assumption that only the slowly exchanging Na is intracellular, the amount of Na leaving the fiber per hour can be calculated. This flux combined with the Na concentration and the resting potential were used for a calculation of the minimum energy required to expel the Na as fast as it enters, assuming no exchange diffusion. This admittedly rather rough estimate gives about 50 Cal/hr/kg. of muscle. According to Conway (15) the resting metabolism of winter frogs' sartorii is about 175 Cal/hr/kg. This means that more than 30 per cent of the energy output of the muscle would be consumed by the active Na extrusion even with a 100 per cent efficiency of the energy transfer from metabolic processes to active transport.

It will thus be seen, that although the Na outflux from the fibers may still be considered identical with the transport, it is rather unlikely that it is so. The possibility of at least some exchange diffusion has evidently to be considered. Thus we are in the situation that we may have already in the isotopes the means for the determination of true transport rates, while on the other hand we may be far from that goal if the exchange diffusion turns out to be a fast process compared with the transport.

Organs which are able to perform a net transport of Na ions (as a rule together with Cl) are widely distributed in the animal kingdom. Well-known examples are the salt reabsorption in the kidney tubules and in the intestine (60, 29, 30). Other examples are the salt secretion in the gills of the eel (68) and the salt uptake from the surrounding water by a wide variety of fresh water animals (79). In most cases the transport seems to go in one direction only. The eel, for instance, which can excrete salt when
in salt water, cannot take up salt from dilute solutions (79). There are, however, exceptions from the rule of one-sided transport. The stickelback (Gasterosteus aculeatus) which was found able to take up salt from fresh water (77) excretes salt when kept in sea water (42). According to Panikkar (105, 106) the brackish water prawn Palaeomonetes varianus which can live in nearly fresh water as well as in sea water is isotonic in water of about 2 per cent NaCl. This species is practically homoiosmotic, the difference in its osmotic pressure over a range of 5 per cent NaCl in the external medium being only 0.8 to 1 per cent. As the urine of this species is nearly isotonic with the blood irrespective of the nature of the medium, Panikkar concludes that the animal must take up salt in dilute medium whereas it excretes salt (and possibly takes up water) in concentrated solutions. A similar ability for osmotic regulation is found in the pacific prawn Metapenaeus monoceros (107).

The ability to transport salt in both directions may turn out to be quite common among crustaceans. Jones (64) thus found that out of nine different species of crabs from the west coast of North America two were able to keep up their osmotic regulation as well in dilute salt water as in salt water more concentrated than the blood of the animals. It has still to be found out whether the salt excretion and the salt uptake are performed by the same cells or whether special organs are present for the two processes. In most of the cases just mentioned the P.D. across the transporting cells has not been measured during the salt transport and therefore it is not quite certain whether it is the Na ion, the Cl ion or both which are transported. There is, however, indirect evidence that a true Na transport is involved in some of the cases of salt transport listed above. The goldfish for example may take up Na not only from NaCl (NaBr) but also from dilute solutions of NaHCO₃ (78). The uptake of Cl is evidently unnecessary for the Na uptake. In one experiment with a frog it even was observed that the animal took up Na for a period of time while simultaneously losing Cl to the solution. A similar independence between Na and Cl uptake has been found to occur quite commonly in the axolotl when kept in dilute salt solutions (4).

As first pointed out by Koch (71) the salt-absorbing organs in a number of arthropods have in common the ability to concentrate Ag ions from very dilute Ag salt solutions. The uptake is indicated by the blackening of the organs (anal papillae of mosquitoes, gills of daphnia and crayfish) performing the uptake, when exposed to light. If the organs are not exposed to light the Ag uptake results in the formation of a white precipitate in the cells which is not AgCl (Koch, personal information). In so far as Ag and Na are both monovalent ions which, judging from the position of the elements in the periodical system are related, one might assume that the Ag simply takes the place of Na in the transport system. Krogh (79) therefore considered it possible that the salt uptake through the anal papillae of the mosquito larvae was in fact a Na transport with the Cl ions following by electric attraction. If this theory is correct one would expect a competition between Ag ions and Na ions in the transporting organs. As a matter of fact Holm-Jensen (56) has found that specimens of Daphnia magna which were killed by Ag poisoning had a considerably reduced Na content; animals killed by organic poisons showed about normal Na content. Poisoning with Cu⁺⁺ or Hg⁺⁺ had the same effect as Ag on the Na content of the animals. The amount of heavy metal sufficient to kill the daphnia was as low as about 0.03
mg/l. The heavy metals only exerted their toxic effect when present as ions, so that complex formers like glutathione in little more than equivalent amounts would eliminate the toxicity of the metal ions. Although these experiments are in agreement with the hypothesis that the heavy metals block the groups which would otherwise bind Na, other possible explanations have to be considered. The very fact that the heavy metals block a number of the metabolic enzymes raises the question whether it is the ion carrier or the system delivering the energy which is affected.

The salt-transporting cells of vertebrates apparently differ from those of arthropods in that they never accumulate Ag. On the other hand there are indications that the salt-transport mechanisms of different organs of vertebrates are related. The fact that the adrenal-cortex hormones influence the Na reabsorption much more than the Cl reabsorption in the kidneys might suggest a specific Na transport mechanism in the kidney tubules. The disturbance in tubular function, following adrenalectomy is shown by an inadequate Na reabsorption from the glomerular filtrate even when the concentration in the plasma is low, and secondly by the failure to excrete K and P at high concentrations when these ions are abnormally concentrated in the blood plasma (43, 87). The normal conditions can be restored by the injection of cortex extracts. Hartman et al. (44, 145) have succeeded in separating from the cortex extract a substance which is highly potent in its ability to cause retention of Na, but having no effect on the K excretion. It occurs in sufficient amount to account for the Na-retaining power of adrenal extract. An interesting point is that this substance binds some Na which is split off as NaCl on treatment with 0.1 normal HCl together with simultaneous loss of a considerable proportion of the potency. Although this Na binding may be coincidental it might be worth while considering whether the Na factor of the adrenals is identical with or is part of the Na carrier in the Na transport system.

The salt absorption in the gut of dogs is also impaired after adrenalectomy (31). The absorption of Na as well as of K and Cl falls after the operation. It is important to note, however, that relative to the Na uptake the K uptake is increased after the operation. In several cases there was even an increase in the Na content of the gut. The experimental conditions were such that the K concentration in the gut at the beginning of the experiment was always higher than that of the blood plasma, whereas Na was taken up against a concentration gradient. It is interesting to note that even the salt uptake through the skin of amphibians seems to depend on the function of the adrenal cortex. It has been found that commercial preparations of the pressor hormone from the posterior lobe of the pituitary induces an increased uptake of NaCl through the skin of the axolotl (3). A considerable net uptake is, as a rule, observed and tracer experiments show a mean of 200 per cent increase in the Na influx. Later it turned out (75) that the adrenocorticotropic hormone from ox pituitaries could bring about a reaction which in all respects resembled that brought about by the pressor preparation. This might indicate that corticotropic hormone present as impurity was responsible for the pressor-fraction reaction. As already mentioned the stickleback can keep a constant salt level in the blood both in fresh water and salt water. In the mating season, however, the regulation mechanism is more or less disturbed and the animals do not stand fresh water or pure salt water very well as
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stated by Koch and Heuts (74). These authors also found that the feeding of thyroxine to this fish brought about severe disturbances in the osmotic regulation if the animals were kept in sea water. The authors assume a disturbance in the normal function of the adrenal cortex to be the underlying reason for the failing osmotic regulation (73).

Only in the case of frog skin do we have definite evidence that a specific Na transport is at work. This organ will therefore be treated at some length.

The presence of a Na transporting mechanism in the isolated frog skin was strongly indicated by Huf's (58) finding that the frog skin will transport salt from the outside to the inside when bathed with Ringer on both sides. Although he did not make Na analyses, Huf evidently considered the Cl transport to be a measure of a salt transport. He speaks of "einer Kraft, die Ionen von aussen—innen bewegt". The potential measurements showed that the inside solution was always positive relative to the outside; Huf did not mention the possibility, however, that the Cl transfer might be due to electrostatic forces. Katzin (67) using the radioactive isotopes Na24 and K42 was able to demonstrate that Na passes with greater rate inward than outward through the frog skin when the same mixture of NaCl and KCl was applied on both sides. The rate inward was 60 per cent higher than the rate outward with pure 0.12 N NaCl on both sides and no less than 300 per cent greater if 80 per cent of the NaCl was replaced by KCl (all the solutions were 0.12 N as to Cl). The experiments with K42 indicated that there was a net movement of K outward, at least at the lower range of K concentrations. The net transfer of Na inward was about 12 to 20 X 10^-8 eq/hr/sq. cm., whereas the net transfer of K outward was 2 to 6 X 10^-8 eq.

Now, it turns out that the inside of the skin is positive relative to the outside under all experimental conditions where a salt transport can be observed; this can only mean that at least the Na ions are transported actively, whereas the Cl ions might follow passively.

Evidence that the frog skin preparations can transport Na, not only against a potential gradient, but also against a steep concentration gradient was given by Ussing (149, 150). The skin was placed as a membrane between two salt solutions (that on the inside being always Ringer's) and the outflux or influx of Na determined with Na24. Concurrently with the flux determinations, the P.D. between the solutions was measured. It was found that the influx is definitely much higher than the outflux even down to about 1 millimolar NaCl on the outside. This means that there is a net uptake of Na in the entire region studied. When the outside concentration is above 10 millimolar, the Na influx may simply be used without serious error as a measure of the net Na transport and we thus have a relatively simple and convenient way of determining the influence of different factors on the transport system.

An interesting point is that the Cl influx is smaller and as a rule much smaller than the corresponding Na influx. This means that part of the Na removed from the outside solution must be replaced by another cation. Preliminary experiments indicate that the ion in question is the H ion. To a lesser extent K (139, 67) and Ca (76) are also lost through the skin. Steinbach (139) found that the frog skin is in equilibrium (as to K) when the solution applied to the inside is 0.0025M in K whereas
the solution has to be 0.01M when applied to the outside. With the same solution on both sides of the skin there will therefore be a constant net outflux of K. This is probably an entirely passive process since the P.D. will force out the K. It is possible that even the Cl ions move passively; in this case the transport of sodium ions across the skin might be considered responsible for the P.D. If both sides of the skin are bathed with the same solution, work has to be done on the Cl ions simply to overcome the frictional forces in the membrane, and as Na is transported faster than the Cl ions can follow, the inside solution will attain a positive charge. If a passive Cl uptake has to take place against a concentration gradient the P.D. required will be that giving the Cl ion the same electrochemical potential in both media plus some excess to overcome the friction. If the outside NaCl concentration is higher than 2 to 5 millimol NaCl and lower than that of Ringer, the P.D. is as a rule found sufficiently high (50–80 mV) to account for a passive Cl transport inward. Simultaneous determinations of influx and outflux of Cl as related to the P.D. would tell whether a special Cl transport mechanism is in action or whether the electric force is solely responsible for the Cl uptake.

To a large extent the behavior of the isolated frog skin toward ions can be explained by assuming that only the Na ion is transported; some evidence is present, however, that in the living frog at least there is also some active transfer of Cl (see below).

The close relationship between Na transport and P.D. is borne out by the fact that they vary in much the same way on changes in the experimental conditions. Especially illuminating is the influence of the pH on Na transport and P.D. Whereas the pH of the outside solution can be varied for instance from 6 to 8 without any effect on Na influx or P.D., both of them show a high degree of dependency on inside pH. If the pH of the inside solution is increased, for example, from 7 to 8 the Na influx increases often as much as three times whereas the P.D. may increase up to 60 mV (the inside becoming still more positive relative to the outside). Meyer and Bernfeld (97) have pointed out that the inside of the frog skin reacts to pH changes much like a glass electrode, giving an increase near the theoretical 58 mV shift for 1 pH unit. They ascribe the reaction to some layer at the inside of the epithelial cells being selectively permeable to H ions. Strickly speaking the H selective layer is also permeable to a number of other ions; but only H⁺ (or OH⁻) participate measurably in the electricity transport. Meyer and Bernfeld assume the P.D. to be brought about in part at least by the formation of H ions within the epithelial cells. These H ions by their diffusion through the H selective layer are assumed to give a positive charge to the inside solution. This explanation cannot be entirely correct because a P.D. brought about by inward diffusion of H ions would give a Teorell effect (147) forcing Na outward. If the H selective membrane hypothesis is to be kept (Meyer and Bernfeld's experiments seem to be conclusive) it can only mean that the Na transport consists in a forced exchange of Na against H ions across the H selective membrane at such a rate that the cellular pH is kept constant (149, 150). Then we would have exactly the same situation as in a glass electrode where the H selective membrane separates a solution of constant pH from one where the pH is varied.

It is interesting to note that the ultimate result of a forced H⁺-Na⁺ exchange
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depends on the permeability of the membrane to the different free ions present. In case the membrane had been just as impermeable to H$^+$ as it is to Na$^+$, no P.D. and no salt transport would have been brought about, but the outside NaCl solution would have been turned into a solution of hydrochloric acid.

The poisoning of frog skin with cyanide stops the active ion transport (58, 150) and parallel with the fall in transport goes a drop in P.D. This indicates the importance of the oxidative metabolism for the transport process. Huf (58) tried to identify still further the reaction responsible for the chloride transport and the P.D. He showed that whereas poisoning with monobromoacetate would stop the transport and depress the P.D., the addition of lactate or pyruvate to the monobromoacetate-poisoned skin could restore, in part at least both P.D. and chloride transport. This result seemed to indicate that the process responsible for the salt transport was connected with the pyruvate oxidation. Francis and Gatty (34), however, have shown that the P.D. of monobromoacetate-poisoned frog skin can also be restored by addition of salts of a number of other organic acids (for instance acetate, propionate and butyrate). Although these authors did not determine whether the substances mentioned were able to restore the salt transport, it is likely that the effect of these acids is similar to that of pyruvate. It thus seems that a number of hydrogen donators can supply the fuel for the transport reactions.

As mentioned above, heavy metals appeared to poison the NaCl uptake in Daphnia (56). Lundegårdh and Burström also found heavy metals to be extremely poisonous to the salt uptake in wheat roots (89). I have therefore made some experiments to find out whether Cu$^{++}$, as a representative of the heavy metals is a specific poison for the Na transport in the frog skin. The result was rather surprising. The presence of as little as $10^{-8}$ moles/l of Cu$^{++}$ in the Ringer bathing both sides of the skin brings about a steady increase in P.D. which after 5 hours may reach values as high as 135 mV, about double the value found for the controls. Later the P.D. declines, but even after 18 hours the skin is still alive as evidenced by a positive P.D. The Cu turned out to bring about a tightening of the membranes which decreased the shortening of the P.D. through the skin. It thus would appear that Cu$^{++}$ rather stimulates the ion transport.

A stimulus may also originate in the nervous system. Steggerda and Ponder (138) found that the injection of very small amounts of strychnine sulfate into living frogs caused the P.D. across the skin to increase violently. In one experiment the P.D. rose from an initial 47 mV to 178 mV 12 minutes after the strychnine infection. It would be very interesting to know how this P.D. change is related to the movements of ions across the skin. The same authors also found that damage to the central nervous system induced a fall in P.D. It is possible that this effect was caused by the release of adrenaline or sympathine from the nerve endings. Barker Jørgensen (2) has shown that adrenaline even diluted $10^{-6}$ will bring about an enormous increase in salt permeability when applied to the inside of the isolated skin. The permeability change is accompanied by characteristic changes in P.D. (149). The use of tracer Na revealed, however, that the adrenaline has still another effect namely to increase the active Na transport. Whether this change in Na influx is primarily due to a stimulation of the cellular metabolism induced by adrenaline or
whether the increase in influx is a secondary effect of the permeability change cannot be decided at present.

The skin is only sensitive to adrenaline applied from the chorion side just as it is far more sensitive to pH changes on the chorion side. This makes it rather likely that it is the basal cell membrane of the epithelial cells which is responsible for the active Na transport. As we have seen already many cells like muscle fibers and nerve fibers must extrude Na through their surface. As far as Na transport is concerned the difference between these cell types and the epithelial cells is that the latter are only extruding Na through the cell membrane turning inward whereas the others do this through their entire surface.

The specificity of the mechanism carrying Na across the skin was clearly demonstrated already by Krogh (76) who found that frogs in need of salt took up Na but not K or Ca from solutions of their chlorides. A most remarkable thing about this transport is that the Na is transported right through epithelial cells having a high content of potassium. Rubin (124) analyzed the skin of *Rana pipiens* for inorganic ions. The K content was 132.4 mg per cent as compared with 90.6 mg per cent of Na. As the chorion is made up mainly of connective tissue with large interspaces containing NaCl but little K, the cells, particularly the epithelial cells, are bound to have a K content which is far higher than their Na content. It is thus safe to assume that Na is transported out through the basal part of the epithelial cells rather than transported into the cells through the membrane turning outward. While the Na is transported in, the K is slowly leaking out.

It may be appropriate to devote a few remarks to the relationship between ion transport and water movement through the frog skin. Already in 1892 Reid (118) observed a very slow fluid transfer across the skin from the outside to the inside when both sides were in contact with Ringer's solution; this observation has since been confirmed by several investigators (59). By some authors this water transfer has been termed irreversible permeability. With heavy water as a tracer, however, Hevesy, Hofer and Krogh (46) were able to demonstrate that the influx and outflux of water are about equal, the net uptake of water being only a small fraction (less than 2 per cent of the total water exchange.

The net uptake of water could be stopped if an isosmotic sugar solution was used as outside medium, so that there could be little doubt that osmosis played a major rôle in the water movement. The net uptake of water was, however, about 5 times the value calculated under the assumption that the influx and the outflux of water (as measured with D₂O) were proportional to the water activities in the medium and in the frog, respectively. At that time, 1935, no really satisfactory explanation of the discrepancy could be given; but later Krogh (79) pointed out that some water may be carried along with the salt which is transported through the skin. Thus there is no reason for assuming any specific water transport mechanism. It is interesting that even in the case of the aqueous humor of the eye, the water exchange is very high compared with the net transfer, so that the water transport can be regarded as the result of the electrolyte transport (69, 70). Parenthetically it should be mentioned that the assumption on which the calculations of Hevesy et al. were based is not absolutely correct. If the water forms a continuous phase through the mem-
brane all diffusing water molecules will have superimposed upon their diffusion rate the mean rate of the water in the membrane; molecules diffusing upstream will be retarded and molecules diffusing downstream will be accelerated. Calculations show (151) that for dilute solutions the net osmotic uptake is about twice that calculated according to Hevesy et al. A similar effect can be predicted for all substances diffusing in the water phase. For small ions at low concentrations the effect is insignificant, however. It is the high concentration of water that emphasizes the importance of this streaming effect. In organs like the gut where a rapid water uptake may take place the streaming effect on the solutes may become important, so that the calculated electrochemical potentials are no longer adequate expressions for the tendency of ions to move in one direction or the other.3

Transport of Hydrogen Ions. It will have been noticed that the transport of potassium into yeast cells might also be called an excretion of hydrogen ions. Similarly the sodium transport through the frog skin could most properly be described as a forced exchange of Na against H. Indeed, most cases of cation transport are probably accompanied with a compensatory transfer of H+ (in some cases perhaps of NH4+). Thus, according to Pitts et al. (110, 111, 112, 113) a forced exchange of Na against H+ is probably responsible for the acidification of the tubular urine. In experiments on dogs it was conclusively shown that, under appropriate conditions, the quantity of acid excreted was much larger than that which had filtered through the glomeruli. This means that either an acid is excreted and its anion reabsorbed together with Na+ or there is simply an exchange of Na+ against H+. Actually these two possibilities can hardly be distinguished from each other. A Na+-H+ exchange would probably require an anionic carrier pendulating back and forth across the cell membrane of the tubule cells, so that, in a way, an acid goes to the tubular urine and a Na salt goes back (134, 125).

The acidification of the urine probably takes place in the distal tubuli as distinguished from the main absorption of NaCl which is performed in the proximal tubuli. At least in the frog (99) the acidification takes place in a short segment near the distal end of the distal convolution. In frogs no acidification is observed if the carbonic anhydrase of the tubuli is poisoned with sulphonamide (53). Hober therefore assumed the acidification to be due to reabsorption of bicarbonate ions. In the experiments with dogs, however, Pitts et al. (111) found that the acid elimination continued although at a slower rate when the carbonic anhydrase was inhibited by sulphonamide. Therefore, according to Davenport (24), it is likely that the carbonic anhydrase has only the function to place the necessary amount of H+ at the disposal of the Na+-H+ exchange system by forming H2CO3 from CO2. Davenport suggests that a forced Na+-H+ exchange is also responsible for the formation of the hydrochloric acid of the gastric juice. The ions originate mainly from the CO2 formed in the parietal cells, but at least in warm-blooded animals the carbonic anhydrase is of little importance for the process because the spontaneous hydration of CO2 suffices to form the necessary amount of H ions. The main work done by the cells is, however, the excretion of the H ions.

3 In the tracer experiments of Vischer et al. (153) there was no simple relationship between the osmotic pressure in the gut and the movements of ions and water.
A very similar hypothesis has been advanced by Conway et al. (17, 18). They also consider the essential process in the formation of the HCl to be exchange of H+ for inorganic cations. A solution of inorganic chloride is supposed to flow past an exchange region and the reabsorbed inorganic cations are returned to the blood stream or to the alkali chloride-secreting part of the cell. By analogy with the ion exchange in yeast these authors assumed the chloride to be KCl, but in a later paper (16) Conway points out that it might be NaCl since a Na transport is known in many other animal cells. The hypothesis of a Na+/H+ exchange being the crucial step in the formation of the acid is in good accord with the known facts about the acid formation. According to Davenport (24) the secretion is in osmotic equilibrium with the blood so that no water transport need be postulated. The fact that Br and, to some extent, I can replace Cl without any effect on the secretion rate speaks in favor of a passive transfer of the chloride ions (25).

Across the wall of the resting stomach there is a P.D. which in frogs is about 50 mV (98, 22) and in dogs 70–95 mV (116). When the secretion of gastric juice sets in (for instance on histamine stimulation) the P.D. drops considerably. In case the stomach wall had been permeable to H+ but impermeable to Na+ the P.D. would have been determined by the proportion between the H+ concentration in the gastric juice and in the blood; \[
\frac{10^{-1.4}}{10^{-7.4}} = 350 \text{ mV}.
\]
We must therefore assume either that the stomach wall is impermeable to H+ or that Na+ diffuses about as fast as does the II ion so that the diffusion potentials nearly cancel out. Tracer experiments with Na have shown that the stomach wall is permeable to Na+ although the permeability in the acid-excreting part is very low compared with the permeability in other parts of the stomach (21). The electrochemical potential of the H ion is evidently very much higher in the gastric juice than in the blood so that an active H+ transport must take place. The fact that the mucosa side is some 20 mV negative relative to the serosa side means that the electrochemical potential of the Cl ion is also somewhat higher in the juice than in the blood, although the concentration of Cl is about the same in serum and gastric juice. This would indicate that an active Cl transport is also involved in the secretory process. However, the P.D. which we can measure is the resultant of the P.D. values across all the cells of the gastric mucosa. If, as there is good reason to believe (86), the acid is only elaborated by the parietal cells, the P.D. across the canaliculi may differ considerably from the 'gross P.D.' which is the only value available to us. The reasonably safe conclusion from the P.D. measurements is that a H+ transport has to take place whereas the Cl− may or may not be transported.

It should be pointed out, however, that the Na+/H+ exchange hypothesis is far from being generally accepted. Despite the enormous amount of work done in the field our knowledge is still so incomplete that a number of widely different hypotheses seem to fit the known facts equally well. Hollander (55) whose article gives a thorough discussion of the relevant literature up to 1943 mentions as a possibility that the hydrochloric acid is formed by membrane hydrolysis of alkali chloride. A contractile mechanism, wholly or in part identical with the pericellular membrane is as-

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* For gastric potentials in human beings see (120).
sumed to exert an intermittent pressure on the cell contents. If the membrane of the canaliculi is taken to be cation impermeable, the Cl ion can only pass out if a corresponding number of OH ions pass in so that, in the end, HCl is formed in the secretion and alkali chloride remains in the cell.

Bull and Gray (12) proposed the interesting hypothesis that a nearly pure solution of some organic acid, such as pyruvic acid, is secreted into the basal end of the canaliculi. As the secretion passes through the canaliculus the anion diffuses back into the cell where the metabolism keeps its concentration at an extremely low level. The H ions cannot follow, however, because the membrane is theoretically cation impermeable. To maintain electroneutrality, Cl\textsuperscript{−} has therefore to replace the organic anions that diffuse away from the secretion.

Other hypotheses where the HCl formation is thought to be brought about by an electric current originating from some metabolic process, have been advanced by Rehm (116, 117) and Davis, Longmuir and Crane (22, 26).

A discussion of the merits and drawbacks of these different hypotheses is hardly warranted because too many unknowns enter into the premises. More work is evidently needed before we can hope to understand the apparently simple formation of HCl from NaCl. The use of new approaches can still bring astonishing results. Thus it may be mentioned that Teorell et al. (85) have found the primary acidity of the secretion to vary between 170 mN and 350 mN whereas hitherto it has been generally accepted that the secretion was isotonic with the blood and of constant acidity.

**ACTIVE TRANSPORT OF ANIONS**

**Transport of Anions in the Animal Organism.** The transport of phosphate in animal cells has been referred to already in the introduction. In this article we shall not enter into a discussion of the transport of organic substances but it should be mentioned that we have examples of manifest anion transport in the excretion of a substance such as diodrast and certain acidic dyes like phenol red in the kidney tubules (54).

A rigorous proof that Cl ions are transported actively in the animal organism is still lacking. We have seen already that the low Cl concentration in muscle and nerve can be explained as the result of a Donnan equilibrium. The uptake of Cl\textsuperscript{−} in many fresh water organisms is as a rule accompanied by a Na\textsuperscript{+} uptake which we have good reason to believe is of active nature, so that a specific Cl\textsuperscript{−} transport need not be postulated. In living frogs, however, Krogh (78) found an uptake of Cl\textsuperscript{−} from KCl, CaCl\textsubscript{2} and NH\textsubscript{4}Cl solutions; K\textsuperscript{+}, Ca\textsuperscript{++} and NH\textsubscript{4}\textsuperscript{+} were not taken up and no significant amount of Na\textsuperscript{+} was present. The Cl\textsuperscript{−} uptake seemed to be an exchange against HCO\textsubscript{3}\textsuperscript{−}. These experiments certainly speak much in favor of a Cl-HCO\textsubscript{3} exchange mechanism. Quite recently Barker Jørgensen and Levi (4) have given substantial support to the idea that under certain conditions some exchange of anions is aiding in the uptake of Cl\textsuperscript{−}. They found that frogs which had stayed in a slow stream of dilute NaHCO\textsubscript{3} for some time showed a net uptake from a NaCl solution of Cl\textsuperscript{−} as well as a total Cl\textsuperscript{−} influx which were well in excess over the corresponding values for Na\textsuperscript{+}. In their experiments with salt uptake in the gut, Ingraham and Visscher (62) observed that the Cl uptake was in excess over the Na uptake in many cases.
At the same time the bicarbonate concentration of the gut content showed an increase. A Cl-HCO₃ exchange would explain this result. The authors point out, however, that the secretion of the intestinal glands contains considerable amounts of NaHCO₃. If Na⁺ and Cl⁻ were taken up in equivalent amounts this would lead to an apparent excess uptake of Cl⁻ in exchange for HCO₃⁻. The secretion of the skin glands of frogs and fishes have not been sufficiently studied to indicate whether enough NaHCO₃ is produced to account for the apparent Cl-HCO₃ exchange. But for the time being the existence of an active Cl⁻ transport in the frog skin cannot be ruled out.

An active concentration of inorganic iodide takes place in the thyroid gland. Also the higher homologue of iodine, ekaiodine is concentrated by the gland (40) and so is to a lower extent bromine (109, 5); but it is not known whether the last two elements are present to any extent as ions or only in inorganic compounds.

Mann, Leblond and Warren (96) demonstrated that about 5 per cent of the I of the thyroid of the normal dog was inorganic iodide. This I could not have been formed from the organic iodine compounds during the isolation process. In experiments where weightless amounts of tracer iodine were given to the dogs it turned out that, at any time, the relative specific activity of the iodine of the diiodotyrosine was higher than that of the free I⁻. This indicates that the major part of the I of the diiodotyrosine does not come from the free iodide of the gland. Possibly the reaction which introduces the iodine in the tyrosine molecule takes place in the surface of the thyroid cells, and at least some of the free iodide of the gland is formed by the splitting off of some of the organically bound iodine.

Later observations are difficult to reconcile with this hypothesis. Thus Leblond (82) found that when large doses of iodide are introduced in a guinea pig the larger part of the iodine fixed by the gland remains in the form of inorganic iodide for some time. Franklin and Chaikoff (36) have found that the formation of radioiodotyrosine and radiothyroxine in thyroid slices is inhibited by sulfanilamide and related compounds in 10⁻⁴ M solution. At this concentration of sulphonamides the capacity of the surviving thyroid slices to take up iodine from the solution was not depressed. It thus appears that for uptake of I there is another route than that over diiodotyrosine.

The thyroids of rats made goiterous by prolonged feeding of propylthiouracil possess the I concentrating mechanism despite a complete block of the formation of organic iodine compounds (146). In fact the capacity of goiterous glands for fixing inorganic iodide is much greater than normal. A linear relation was found between the iodide concentration in the thyroid and plasma, the concentration factor being 200 to 300. The uptake of iodide by the goiterous gland is inhibited by KCNS (146, 152); but the CNS ion is but little concentrated if at all. Although the iodide might exist in the gland in a very loosely bound form (128), it is just as likely that an active transfer of iodide takes place and that it is the transport mechanism which is blocked by CNS. The presence of the I in free rather than in bound form would explain that the iodide is freely dialysable and ultrafiltrable and that it is not coprecipitated with the protein of the gland when the tissue is homogenized with protein precipitants (146).

Transport of Anions in Plants. 1) The ion uptake in plant roots. Excised roots of young plants of the grass family possess a pronounced ability to take up ions even
from dilute solutions. This process has been studied very carefully by different
groups of investigators, but so far no unanimously accepted view has been obtained
as to the mechanism involved. Lundegårdh, as well known (90, 92, 94) assumes
that the process is essentially an anion uptake whereas the cations are supposed to be
electrically attracted. Hoagland and Steward (49, 50), on the other hand, do not
consider Lundegårdh’s theory convincing and they apparently prefer the assumption
of a cation transport as well as an anion transport. As a matter of fact the ion up-
take observed is the resultant of two different phenomena, namely an ion accumula-
tion in the cell sap of the roots’ parenchyma and a transport of ions from the outside
solution to the wood vessels, where they contribute to the formation of the bleeding
sap. Although we do not know whether both of these phenomena are brought about
by the same mechanism, there seems to be a rather close correlation between them.
Hoagland and Broyer (48) have shown that the concentrations of K, Na and Br in
the cell sap and bleeding sap from barley roots vary in very much the same way with
time, when the roots are transferred from distilled water to an experimental solution
containing these ions. The ion concentrations are, however, always higher in the cell
sap than in the bleeding sap. Naturally a formal similarity in the rates of ion uptake
in cell sap and bleeding sap is not sufficient to prove that both processes are due to
a common mechanism. Especially Arisz (1) has stressed the importance of treating
the transfer across the cell membrane and across the tonoplast separately. The
potential measurements which have been performed by Lundegårdh (91, 92, 93) and
others (130) only relate to the P.D. between cell sap and bleeding sap. We are with-
out any direct knowledge as to the P.D. between cell sap and protoplasm as well as
to that between the epidermal cells and the medium. Lundegårdh has not stressed
this point sufficiently. In fact he assumes that the whole potential difference is
located at the boundary between the epidermal cells and the medium (93). In view
of the fact that a number of cell membranes, each the possible seat of a P.D., are
interposed between the outer membrane of the epidermal cells and the bleeding sap,
this assumption is not very well founded. Lundegårdh himself (94) believes that
there is an anatomical-physiological differentiation of the root parenchyma so that
the power of ion uptake declines from the outside inward. Such a differentiation
most likely would involve potential differences between the cells. For the time being
the estimation of these potentials is hardly technically possible. Nevertheless
Lundegårdh’s potentiometric studies are of great value for our present inquiry as to
which ions are actively transported; but we have to confine this discussion to the
transport from medium to bleeding sap. The latter (94) is a dilute salt solution
practically free of organic matter. The dominating ions are K$^+$ and NO$_3^-$ which also
are the ions most readily taken up from the medium; but almost any ion which is not
too poisonous is taken up. As already shown by Laine (81) the concentration of any
cation in the bleeding sap depends on the concentration of that ion in the medium in
a way that recalls Freundlich’s adsorption isotherm. The salt concentration in the
bleeding sap is higher than that of the medium at low outside concentrations, but as
the outside concentration is increased the relative increase in the bleeding sap be-
comes less and less. We may take as an example one of Lundegårdh’s experiments
(94). It turned out that below about 30 millimoles KNO$_3$ in the medium the bleeding
sap is more concentrated as to K$^+$ and NO$_3^-$ than the medium. With a one milli-
molar solution the KNO$_3$ has been concentrated about 13 times. It is quite evident that work has been done on the NO$_3$ ion. Not only has it been concentrated, but the concentration has taken place against a P.D. which may be estimated to have been some 52 mV (91). This P.D. on the other hand would suffice to account for at least the better part of the K$^+$ concentration. About 65 mV would give nearly equal electrochemical potential in medium and bleeding sap. In view of the fact that the data are taken from different experiments the accordance between theory and experiment should not be stressed too much; but it seems not unreasonable that the K transport is due to electrical forces. The P.D. of the roots is primarily determined by the pH of the outside solution (91, 92). This suggests a permeability to free H ions which is much higher than the permeability to other ions. Moreover the P.D. depends on the outside salt concentration, decreasing from 150 to 200 mV in distilled water to about 60 mV in a millimolar solution of KCl or KNO$_3$. Whatever the origin of the P.D. it is obvious that it suffices or at least contributes substantially to bring about the cation uptake even from very dilute solutions.

In the cytoplasma and the cell sap, however, the K$^+$ concentration is much higher than in the bleeding sap. It is clear that if the K$^+$ concentration is brought about by electric attraction, the P.D. between medium and cell sap must be considerably higher than that between medium and bleeding sap. Considering the fact that certain plant cells like yeast and Valonia are known to concentrate K$^+$ actively it is hardly justified to take definite standpoint as to whether the high K$^+$ concentration in the cell sap is also due to anion transport or whether a specific K$^+$ transport is responsible.

Shuffelen and Loosjes (130) have derived a formula expressing the cation uptake in roots as a function of the concentration and the P.D., with a correction for the ions that are adsorbed to the root surface. The formula seems to be in good accord with the experimental data. As, however, the uptakes refer to the total root and the P.D. is measured according to Lundegårdh and Burström between the cut end of the root (bleeding sap) and the medium, the verification of the formula must either be incidental, or, more likely the P.D. between bleeding sap and medium must be closely correlated to the P.D. between cell sap and medium.

The space does not allow a detailed discussion of the vast number of papers concerned with salt uptake in plant roots. The reader is referred to the excellent general treatments of the subject given by Hoagland (47), Arisz (1), Lundegårdh (94) and Steward (141). Only some points which may have a bearing on the transport mechanism will be presented.

The ion uptake is generally considered as a strictly aerobic process, although a small and irregular anaerobic ion uptake has been reported (94). Cyanide in low concentrations prevents salt accumulation in barley roots (48) and in wheat roots (89). An interesting property of the transport system is that methylene blue destroys the power of salt accumulation without decrease in CO$_2$ production (48). These facts are taken by Hoagland and Broyer (48) to indicate that the salt accumulation is linked with a metal catalyzed aerobic respiration system. A similar viewpoint was also advanced by Lundegårdh and Burström (89). Lack of oxygen stops the uptake of salts as well as of water in barley roots (48). This does not prove definitely, however, that the transport mechanism can only work in the presence of
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molecular oxygen. By favoring fermentation low oxygen pressure brings about an increased formation of organic acids with a resulting decrease in pH, and low pH is known to lower the permeability of many cell membranes (7, 108, 102). A number of observations show that a high CO₂ tension (causing a fall in cellular pH) will also depress the uptake of salts and water in roots (13, 48).

The ion transport in plant roots shows a low degree of specificity. A large number of different anions and cations are taken up. If the cation uptake is due to electric attraction it is no wonder that the uptake is mainly determined by the size and charge of the ions. But even the anion transport shows hardly any specificity although for instance NO₃⁻ is taken up by wheat roots about 4 times faster than SO₄²⁻ (90).

The metabolism of the roots shows a pronounced dependence on the rate of salt uptake. As well known Lundegårdh and Burström (89, 90) assume the increase in metabolism to be determined mainly by the amount of anions taken up. This view has not been generally adopted, however. It seems certain that by a process as yet unknown, the amount of cations taken up (especially K) determines the rate of formation of organic acids (mainly malic acid) in the cells. This acid formation is so regulated that the cellular pH is maintained fairly constant (47).

2) Salt Uptake in Halicystis. Using a very elegant perfusion technique Blinks (7, 8) has been able to make electrometrical studies of the protoplasm-sap interphase as well as of the outside surface of this marine alga. Exact chemical analyses of the protoplasm are not available, but it is assumed that the Cl ions are to a large extent replaced by organic acids. The P.D. between the sea water and the protoplasm may thus be regarded mainly as a Donnan potential, Cl⁻ being the only penetrating anion. In the sap the organic acids are virtually absent. Blinks assumed, however, that “there is an active accumulatory mechanism which is constantly aiding the depletion of chloride from the protoplasm which is only a way station towards the vacuolar depot.” Although the Cl⁻ concentration is only little higher in the sap than in the sea water the maintenance and increase of this Cl⁻ concentration requires work because there is a constant P.D. of about 70 mV to be overcome. Ultimately this Cl⁻ transport should probably be held responsible for the P.D. between the outside and the lumen when both membranes are in contact with sea water.

Whether or not there is also a cation transport cannot be said with certainty. The P.D. in H. ovalis seems to be sufficiently high to account for the high K⁺ concentration in the sap of this species, and H. Osterhoutii does not concentrate K⁺ at all. A number of factors influence the P.D. and presumably the ion transport in Halicystis. The P.D. falls to very low values when the organism is subjected to low O₂ tension. At the same time the electric resistance of the membranes increases violently. A similar reaction is brought about by weak acids like CO₂ and acetic acid and by prolonged stay at low temperature. In all cases, according to Blinks (7, 8) the explanation seems to be a fall in membrane permeability, brought about by a more acid reaction of the protoplasm. This behavior of the cell membranes makes it very difficult to decide whether the ion transporting mechanism is in itself strictly aerobic or whether the fall in transport at low O₂ tension is simply due to permeability changes.

3) Ion Uptake in the Characeae. As already mentioned (104) the cell sap of the
fresh water characean *Nitella* is normally negative relative to the outside solution. When placed in a solution containing 9 millimoles NaCl and one millimole KCl/l, the P.D. was estimated to be about 100 mV. The high concentration of cations, notably K\(^+\) in the sap may therefore be the result of electric attraction, whereas the anions of which the chloride ions make up the dominating fraction have to be transported actively. *Nitella* can live and still take up salts from solutions which are much more dilute as to salts than that mentioned above. It cannot be decided for the time being whether the P.D. of the cells in these very dilute solutions will still suffice to bring about the cation uptake. In their study of the permeability of the membranes of *Nitella* and *Tolypellopsis* Holm-Jensen, Krogh and Wartiowaara (57) consider the exchanges of K\(^+\) and Na\(^+\) ions to be due to an active transport into the protoplasm and a passive diffusion out. Although this interpretation of the results may be correct the possibility of passive diffusion between protoplasm and surroundings cannot be ruled out. The interesting attempt made in this paper (57) to calculate the relative permeabilities of the outer and inner plasma membranes are based on the assumption that the diffusion rates are solely determined by the concentration gradients of the ions; whether or not the P.D. suffices to bring about the cation uptake, the potentials will certainly modify the diffusion rates. If, as Osterhout (104) has found, the main part of the P.D. is between plasma and cell sap it might be suggested that at least part of the anion transport takes place across this structure. Hoagland and Broyer (48) in experiments with radio-Br found this ion to approach a higher activity in the cell sap than in the plasma; this they take to indicate a secretory process transferring Br (and Cl) from the protoplasm to the sap.

**CONCLUSION**

It has been one of the main objectives of the present paper to point out that specific chemical reactions between certain inorganic ions and organic cell constituents is of decisive importance for the ion transport processes. Although the pore size of the membranes and electric potentials resulting from metabolic processes are bound to play a major part in determining the ultimate result of the specific transport processes, pores and potentials cannot alone explain the ion distributions found.

The problem of specific ion transport should conceivably be attacked along similar lines to those used to characterize the metabolic enzymes, so that we must find specific poisons for the different steps involved. But for the time being the major problem remains to determine which ions are actively and specifically transported. The study of the cellular metabolism has taught us a lesson which is well worth remembering even in the study of active transport. What seemed to be a simple combustion of organic matter turned out to be the resultant of an enormous number of enzyme reactions. Similarly, the number of ion transport mechanisms may also be very large; but in certain organs some process or other may be vastly superior to the others in determining the ion movements and in such organs the particular systems may be studied.

It is the belief of the author that the combined use of tracers and electrophysiological techniques will prove very useful to determine which specific transport processes are predominant in different cells and tissues.
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