PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Introductory Remarks

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Introductory remarks

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[Plate 23]

It is a generally accepted viewpoint that the processes underlying active transport in tissues are basically the same as those which are operative in individual cells. However, the organization of cells to form epithelia seems to endow them with new potentialities with respect to apparently active transport of solutes and water not usually recognized in individual cells. The new potentialities probably can be traced back to two main sources: (1) that the cells in an epithelium are polarized so that the 'inward' and 'outward' facing membranes have different properties, and (2) the presence of a system of interspaces which is usually more or less closed towards one side of the epithelium and more open toward the other. In principle the interspace system allows relatively rapid bulk flow which in turn may give rise to 'solvent drag', 'anomalous solvent drag', and in specialized structures even to counter-current interactions between different streams.

General introduction

It is with the greatest anticipation that I have come to this meeting. The programme encompasses papers on transport in organism of the most diverse types, from humble plants over squids and insects to the elevated level of vertebrates. I have no inside knowledge as to the thoughts underlying the composition of the programme, but it looks to me as if Dr Keynes has intended to widen our scope, not allowing us to become satisfied with a single model for transport which may seem to fit our favourite experimental object. Man has such a strong liking for simplification that only a constant barrage of adverse experimental results can keep him from advancing sweeping generalizations. From my own experience I remember the time when we were collecting evidence for the hypothesis that active sodium transport were almost exclusively responsible for the electric potential difference across the isolated frog skin. At the same time Hodgkin and collaborators discovered the importance of active sodium extrusion as the charging device for nerve and several groups including our own were coming to agreement as to the importance of sodium transport in the ionic balance of muscle. It seemed very tempting to see the sodium pump as the mechanism behind all bioelectric potentials, at least in the Animal Kingdom. Thus I confess that I was rather surprised and maybe a little disappointed when Adrian Hogben, working in our laboratory, found active chloride transport to be responsible for the gastric potential. The existence of a multiplicity of pumps was commonly accepted in the fifties, but the 'unit-pump' idea had a revival when, though the work of Skou (1960), and others the sodium/potassium activated ATPase appeared on the scene and when it was discovered that the transport of sugars and amino acids might be coupled to sodium transport (for references, see Crane 1965).

It is obvious that systems exist which are completely independent of the 'common' sodium pump. Not only micro-organisms and plants, but also insects present a refreshing variety of transport systems which do not seem to fit into the unifying scheme. One may still speculate as to whether or not a common denominator for all transport systems exists, but it has yet to be proposed.

It is apparent that most of the papers to be presented at this meeting deal with transport through epithelia.

In a period of the study of transport phenomena which is now history the study of transport through epithelia served the purpose of demonstrating beyond any reasonable doubt that active transport does exist. Today the existence of active transport even in individual cells is no longer questioned, and as far as the study of the molecular mechanisms goes, transport in cells like erythrocytes and giant nerve fibres offers great advantages compared to similar studies on epithelia. Today the studies on epithelia tend to centre around the things which epithelia can and individual cells cannot do. Most workers in the field believe that the processes underlying active transport in tissues are basically the same as those which are operative in individual cells. However, the organization of cells to form epithelia seems to endow them with new potentialities with respect to apparently active transport of solutes and water not usually recognized in individual cells. The new potentialities probably can be traced back to two main sources: (1) that the cells in an epithelium are polarized so that the 'inward' and 'outward' facing membranes have different properties, and (2) the presence of a system of interspaces which is usually more or less closed toward one side of the epithelium and more open toward the other. In such a system where diffusion equilibration is slow relative to the bulk flow, the phenomena designated 'standing' gradients', 'solvent drag', 'anomalous solvent drag', etc., become of importance, and in specialized structures one may even see counter-current interactions between different streams.

My allotted time does not permit me to discuss all the interactions which may arise from the organization of cells into epithelia, but some of them will be discussed by other participants in the meeting. However, I should like to report in some detail on one particular transport anomaly which we have met in the isolated amphibian skin. It exemplifies the class of new phenomena one encounters when passing from organizational level of single cells to that of epithelium.

Anomalous transport in frog skin, induced by osmotic gradient

Several years ago we observed (Ussing & Andersen 1956) that the electric resistance of the frog skin decreased violently when the osmolarity of the outside bathing solution was increased above that of the solution bathing the inside. The phenomenon was later studied in more detail (Ussing & Windhager 1964; Ussing 1966). Two interesting facts emerged from these studies: first, it was the existence of an osmotic gradient and not the osmotic pressure as such which was responsible for the drop in resistance, since the electric properties of the skin returned to normal values if the osmotic pressure was also raised on the inside. Secondly, the drop in resistance was due to a reduction of the skin potential whereas the short-circuit current was much less affected. Thus it was tempting to assume that the osmotic gradient created a shunt-path which would allow chloride ions and other passive ions to short-circuit the sodium battery. One possibility immediately offered itself, namely, that the osmotic gradient led to an opening of the 'tight seals' or zonulae occludentes between the outermost layer of living cells in the epithelium.

One consequence of this hypothesis is that sodium which was transported inward by the sodium pump might to some extent escape 'backward' through the leaky seals, so that some sodium would recycle through the pump. This in turn might lead to reduced efficiency for the pump so that the constant ratio (Zerahn 1956; Leaf & Renshaw 1956) between the number of

moles of sodium transported and the number of molecules of oxygen used for transport should be offset. This turned out to be the case (Ussing 1966). Thus the working hypothesis looked promising. To complete the picture we wanted to demonstrate that the leak created by an osmotic gradient would allow a test substance like sucrose to pass the epithelium. Sucrose was chosen, because it is known not to penetrate at all easily into animal cells. The first experiment looked like a great success. With Ringer's solution on both sides of the skin, the permeability to sucrose was insignificant, but increased by a factor of 20 when 200 mmol/l of urea was added to the outside solution. However, we got a surprise when the influx of sucrose were studied in parallel experiments. It turned out that the influx was always larger than the efflux. It soon became apparent that the transport anomaly was of a very general nature: not only sucrose but also other sugars as well as inorganic ions like chloride, sulphate and sodium underwent anomalous inward transport; furthermore, the osmotic gradient necessary could be produced by addition to the outside medium of a wide variety of osmotically active substances, including sodium chloride. It also became clear that although solutes like sucrose were carried inward (opposite to the expected direction of solvent drag) there was a net transfer of water in the outward direction. The phenomenon of anomalous transport induced by an osmotic gradient was also observed by Franz & Van Bruggen (1967). These authors proposed that the phenomenon were due to 'solute drag', that is an interaction between two kinds of solute molecules in the membrane. Originally we assumed that the anomaly was a consequence of the active sodium transport rather of the type which has been proposed by Diamond (1962) for coupled transport in the gall bladder. Further experiments showed, however, that the anomalous sucrose transport in the frog skin could be brought about in the complete absence of sodium in both outside and inside solution. Under such conditions there is no potential so that also electro-osmosis could be ruled out. Thus it appeared that the osmotic gradient was the source of energy for the transport of the test molecule. The immediate interaction between the 'driving' and the 'driven' species of solute seemed unlikely, however. Any medium-sized molecule, independent of chemical nature and charge, seemed to be able to drive any other molecule. Therefore it seemed more likely that the coupling were mediated by the solvent. This could require that the osmotic gradient creates local anomalous osmosis of the solvent which in turn exerts anomalous solvent drag upon the 'driven' species. Such a coupling can be brought about in different ways. One possibility is shown in figure 1.

Tentative model for anomalous transport in frog skin

Urea will diffuse from the outside compartment into the space between the cells through the 'leaky seal' represented by the dotted line. Once in the interspace the urea will increase the osmotic pressure locally and thus will draw in water from the cells. This results in an increase in hydrostatic pressure in the interspace, and the excess urea solution will drain off, mainly toward the right into the inside bathing solution because the flow resistance is less in this direction. The water lost by the cells due to osmotic suction of urea will be replenished by osmosis mainly through the inward facing cell membrane because the outward facing cell membrane is rather tight to water (MacRobbie & Ussing 1961). It is now clear that any sucrose molecule (or other molecule, for that matter) which happens to diffuse into the interspace from outside will be dragged inward by the solvent flow, whereas a molecule coming from the inside solution will be slowed down. The model explains why small

molecules like urea give rise to asymmetric transport, whereas large molecules like raffinose do not (Franz & Van Bruggen 1967). The mechanism will obviously work only if the reflexion coefficient for the 'driving' molecule is low at the left-hand opening between the cells (the dotted line). For large molecules one should expect normal osmosis and an outward directed solvent drag.

It is also necessary that the reflexion coefficient for the 'driving' substance be high at the boundary between cells and interspace. This is not unreasonable in the case of urea which is known to penetrate into the frog-skin epithelium cells rather slowly.

One consequence of this model is that a hydrostatic pressure head on the inside of the skin should slow down the flow in the interspace between the epithelial cells or perhaps even reverse the direction of flow. This again should abolish the anomalous solute transport. Actually we were able to show that a moderate pressure head of 15 to 25 cm of water on the inside sufficed to turn a net inward transport of galactose into an outward transport. One could object that

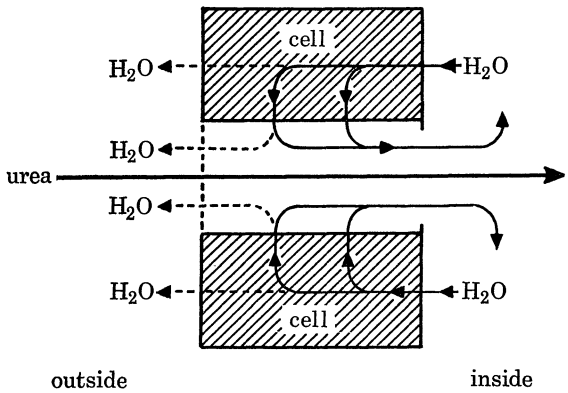

FIGURE 1. (Modified after Ussing 1969.) Schematic picture, showing the origin of anomalous solvent drag in an epithelium where the 'tight seals' toward the outside has become somewhat leaky. The vertical dashed line between the outward facing (left) ends of the two cells is assumed to be nonselectively permeable to all small and medium-sized molecules, but presenting a definite resistance to hydraulic flow. The cell membranes, on the other hand, are assumed to exhibit a high reflexion coefficient, even to urea. The interspace between the cells is relatively open toward the right via large pores in the basement membrane.

the pressure head created new pores, but certainly in the absence, of an osmotic gradient, the skins were practically tight to sugars, whether or not there was a small pressure difference across it.

LOCALIZATION OF TRANSPORT PATH BY $BaSO_4$ PRECIPITATION

Quite recently we have tried an even more direct way of demonstrating that the main transport path created by osmotic gradients is intercellular. The method depends on the fact that if Ba^{2+} is added to the outside and SO_4^{2-} to the inside bathing solution, $BaSO_4$ will precipitate out where the two ions meet (Ussing 1970). Ba^{2+} at a concentration of 10 mmol/l has no appreciable effect for hours when added to the outside of a normal skin, and sulphate is tolerated very well indeed on the inside. Usually no precipitate is formed for hours as long as the solutions bathing the skin are of the same osmolarity or if that on the inside has the higher osmolarity. But if the osmolarity of the outside solution is increased, by say, 100 %, through addition of

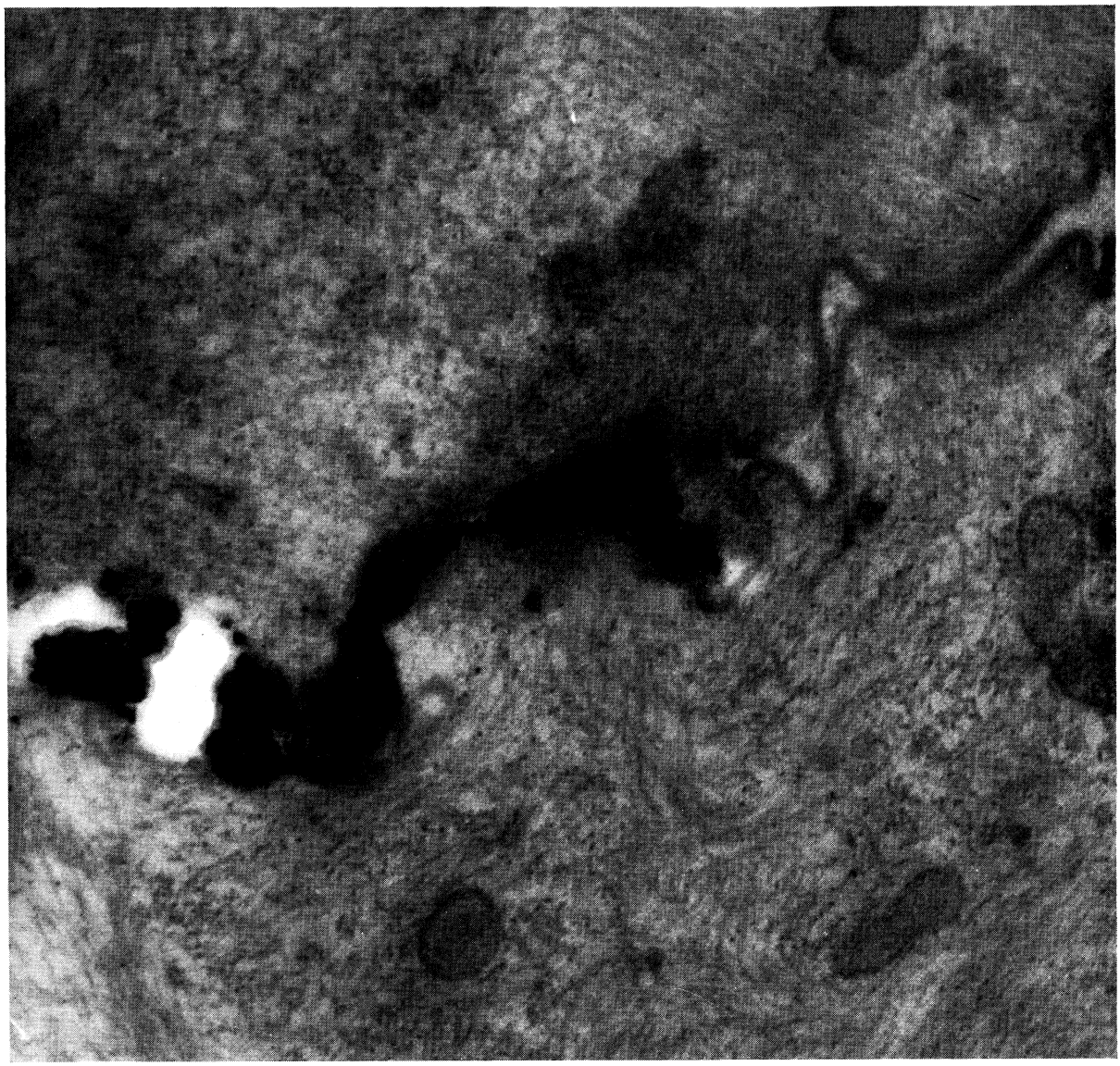

FIGURE 2. Electron micrograph showing barium sulphate precipitate in frog skin. The crystals are precipitated exclusively in the intercellular spaces of the epithelium. Initially frog's Ringer with 10 mmol/l barium chloride on outside and equal parts of chloride Ringer's and sulphate Ringer's on inside. No precipitate visible. After addition of 200 mmol/l of urea to outside solution precipitation takes place. From Ussing (1970).

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urea or NaCl, $BaSO_4$, crystals start forming in the interspaces. Figure 2, plate 22, shows an electron micrograph of the epithelium of *Rana temporaria* with $BaSO_4$ crystals in the interspaces and none in the cells. Such observations in connexion with the fact that hypertonicity of the outside bathing solution increases the permeability to both barium and sulphate in both directions makes it highly unlikely that the cellular pathway is of importance for the passage of these two ions. It seems then that a model like the one depicted in figure 1 is in agreement with most of the observed facts.

One may conclude from the foregoing that the tight seals in the frog skin epithelium are rather tight. In a few cases when the experiment was extended over several hours, we have, however, observed $BaSO_4$ crystals in the interspaces of control skins (without an osmotic gradient). Thus, probably, the kind of leakiness produced by an osmotic gradient is different only in degree and not by principle from the leakiness of low-potential skins.

But why is it that the 'tight seals' open up when there is an osmotic gradient in the outward direction and close when the direction of the gradient is reversed? Such an osmotic rectification can be produced in several ways. Thus a sandwich of two membranes with different water permeabilities will be pressed together when the gradient is in one direction and driven apart when the gradient is reversed. Instead of accepting such an *ad hoc* explanation it may be more profitable to consider the known properties of the 'tight seals'. On the one hand they must possess a variable leakiness in the direction perpendicular to the epithelial surface. On the other hand, according to Lowenstein and collaborators (see Loewenstein & Kanoe 1964) the zonulae occludentes serve as coupling devices between neighbouring cells, allowing small ions and sometimes even large dye molecules to pass from one cell to the next. The geometrical solution for a structure which allows two diffusion streams to pass each other without mixing in directions perpendicular to each other is some kind of meshwork. We shall now make three assumptions: (1) Osmotically active materials diffuse readily between the epithelial cells and the material forming the meshwork of the junctions. (2) The osmolarity of the cells is at all times close to that of the inside bathing solution (which means that the main barrier to osmotic flow is the outward facing membrane of the cells). (3) When the material forming the meshes is exposed to a hypertonic solution it shrinks, facilitating passage through the meshes in the direction perpendicular to the plane of the epithelium.

It is readily seen that such a system at least qualitatively would show the desired properties. So far, of course, the proposed structure of the tight seal is rather speculative. To my knowledge, the superfine structure of the tight seals of the frog skin is not known. The freeze-etch studies of the intestinal epithelia of mice by Staehlin, Mukherjee & Williams (1969) do indeed indicate that the tight seals are based upon an intricate meshwork interposed between neighbouring cells, but it may be premature to try explaining the function of the many interesting details which they have brought to light in terms of the working hypothesis, advanced above.

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FIGURE 2. Electron micrograph showing barium sulphate precipitate in frog skin. The crystals are precipitated exclusively in the intercellular spaces of the epithelium. Initially frog's Ringer with 10 mmol/l barium chloride on outside and equal parts of chloride Ringer's and sulphate Ringer's on inside. No precipitate visible. After addition of 200 mmol/l of urea to outside solution precipitation takes place. From Ussing (1970).