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# Some 'Partial Reactions' of the Sodium Pump

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*Phil. Trans. R. Soc. Lond. B* 1971 **262**, 91-102
doi: 10.1098/rstb.1971.0080

*Phil. Trans. Roy. Soc. Lond.* B. **262**, 91–102 (1971) [ 91 ]
*Printed in Great Britain*

# Some 'partial reactions' of the sodium pump

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The lecture describes recent work on a number of different partial reactions involving chemical transformation, and discusses the relations between these reactions and the movements of sodium and potassium ions. An account is given of recent experiments from the physiological laboratories at Yale and at Cambridge suggesting that ADP as well as ATP is required for sodium–sodium exchange, and that nucleotide is also required for potassium–potassium exchange.

The phrase 'partial reactions' is, of course, borrowed from those who work on mitochondria, and it is used here to refer to various kinds of behaviour which may be attributed to the sodium pump machinery, and which are of interest because they may help to elucidate the nature of that machinery.

Various partial reactions are now known, and they have been discovered from experiments with two rather different kinds of preparation. A tissue may be ground up, and the fragments of cell membrane studied by the sorts of technique the biochemist generally uses to study soluble or particulate enzymes. In this way it is possible to observe chemical reactions, to determine what substrates, co-factors and so on, are necessary for these reactions, and even to identify intermediates in the reaction sequence. But to study an ion pump it is also necessary to be able to follow ion movements, and this can be done only in intact cells. If, at the same time, the investigator wishes to be able to control conditions on both sides of the cell membrane, he is virtually restricted to working either with resealed red cell ghosts—where substances can be introduced into the cells by adding them to the lysing solution—or with cells like the giant axon of the squid—where it is possible to inject directly into the cell.

In the last few years it has become clear, in red cells at any rate, that the entire pump system is reversible, so that, if the supply of energy is not too high and the concentration gradients are even steeper than normal, the whole system runs backwards synthesizing ATP at the expense of downhill movements of sodium and potassium (Garrahan & Glynn 1967*d*). When the pump is running forwards, something like three sodium ions are pumped out and rather less than three—perhaps two—potassium ions are pumped in, for each molecule of ATP hydrolysed (Post & Jolly 1957; Garrahan & Glynn 1967*c*). The stoicheiometry when the pump is running backwards is more difficult to determine, but for potassium at least it does not seem to be very different; something like two or three potassium ions move outwards for each molecule of ATP synthesized (Glynn & Lew 1970).

If the pump can run in either direction, we may expect to find partial reactions that represent steps in either direction, or even a combination of steps in both directions. Let us look at some of these reactions.

### Reactions observed in fragmented membranes

One kind of behaviour that is extremely well established, having been seen in half a dozen laboratories and in at least four different tissues, is observed when fragments of cell membrane with (Na + K)-dependent ATPase activity are exposed to $\gamma^{32}$P-ATP. In the presence of magnesium and sodium ions (but without potassium), there is an incorporation of radioactivity into the fragments; if then the fragments are exposed to potassium ions—and at this stage neither magnesium nor sodium is necessary—the radioactivity is released as inorganic phosphate (Post, Sen & Rosenthal 1965; for further references see Glynn 1968). It looks as if a transphosphorylation reaction requiring sodium and magnesium ions is followed by hydrolysis activated by potassium ions.

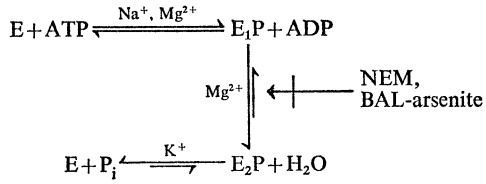


Figure 1. Scheme to explain requirements for ATP–ADP exchange. Modified from Siegel & Albers (1967).

The radioactive phosphorus incorporated into the membrane fragments is found in the lipoprotein fraction; it has a number of properties suggesting that it exists as an acyl phosphate, and Kahlenberg, Galsworthy & Hokin (1968), using a combination of proteolytic digestion and chromatography, have shown that the acyl phosphate is almost certainly a $\gamma$-glutamyl phosphate.

Since the free-energy of hydrolysis of an acyl phosphate is likely to be high, the transphosphorylation reaction that occurs in the presence of sodium and magnesium ions ought to be reversible. The incubation of membrane fragments with ATP and labelled ADP in the presence of magnesium and sodium ions should therefore lead to the incorporation of some of the label into the ATP. In experiments on a membrane preparation from the electric organ of the electric eel, Albers and his colleagues (Fahn, Koval & Albers 1966; Fahn, Hurley, Koval & Albers 1966; Siegel & Albers 1967) were able to demonstrate a sodium-dependent ATP–ADP exchange reaction, but they could demonstrate it only under two rather odd conditions. Either they had to work with very low, but not zero, concentrations of magnesium ions, or at more usual magnesium concentrations they could detect the exchange reaction if the membrane preparation had been pretreated with *N*-ethylmaleimide (NEM) or with an equimolar mixture of arsenite and 2,3-dimercaptopropanol (BAL-arsenite). To explain these curious requirements, they suggested that the phosphorylated compound first formed from ATP, which they called $E_1P$, was converted to another form, $E_2P$, by a reaction which required magnesium ions in moderately high concentration and which was blocked by NEM or BAL-arsenite. The equilibrium for the interconversion of $E_1P$ and $E_2P$ was, they supposed, markedly in favour of $E_2P$.

If figure 1 correctly represents the situation, very low concentrations of magnesium, or pretreatment with NEM or BAL-arsenite, should lead to the accumulation of $E_1P$ and therefore to the occurrence of ATP–ADP exchange; with normal magnesium concentrations and without pretreatment with sulphydryl reagents, $E_1P$ would be transformed to $E_2P$ and little exchange could occur. In support of this hypothesis, Siegel & Albers showed that when preparations pretreated with BAL-arsenite were exposed to $\gamma^{32}$P-ATP in the presence of sodium and

magnesium ions, radioactivity was incorporated into the membrane in the usual way, but this radioactivity was not released by potassium ions. Further evidence in support of the scheme comes from the experiment of Post *et al.* (1969) illustrated in figure 2. A kidney membrane preparation was allowed to incorporate $^{32}P$ from $\gamma^{32}P$-ATP in the presence of sodium and magnesium ions, and then, at a time corresponding to zero time in the figure, a large amount of unlabelled ATP was added. Since any further incorporation would have been of unlabelled phosphorus, the subsequent loss of radioactivity from the membrane fragments gave a measure of the rate of breakdown of the intermediate. With the normal preparation, potassium ions

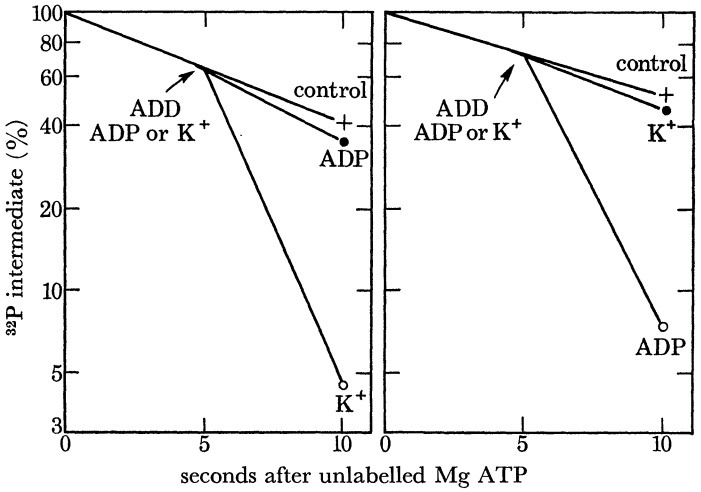


FIGURE 2. The effects of K ions and of ADP on the stability of the phosphorylated intermediate in an untreated membrane preparation (left) and in a preparation pretreated with NEM (right). (From Post *et al.* (1969). Reprinted with permission from *J. gen. Physiol.* **54**, 306s.)

greatly accelerated the breakdown, whereas ADP had only a small effect. With the NEM-treated preparation, ADP caused a rapid breakdown and potassium ions had little effect. This is precisely what the scheme in figure 1 predicts.

Although $E_1P$ and $E_2P$ clearly have different properties, treatment with pepsin yielded products with identical electrophoretic behaviour (Post *et al.* 1969). The difference is therefore likely to be a matter of configuration or position or accessibility rather than of basic chemical structure.

## Na–Na EXCHANGE

With the scheme of figure 1 in mind we are in a position to look at some partial reactions involving the movement of ions. If red cells are incubated in a physiological salt solution, they pump sodium out and potassium in; but if there is no potassium outside, the pump mechanism catalyses an exchange of internal for external sodium ions (Garrahan & Glynn 1967*a*). Under certain conditions a similar shuttling of sodium ions takes place across the membrane of the giant axon of the squid (Baker *et al.* 1969). Although a one-for-one exchange of ions need not consume any energy, the exchange does not occur in the absence of ATP (Garrahan & Glynn 1967*b*; Baker *et al.* 1969); nevertheless, in red cells—and there is no evidence on this point from experiments on the axon—rather little hydrolysis of ATP is associated with the exchange (Garrahan & Glynn 1967*c*). The question then arises, why is ATP necessary?

There seem to be two possible answers. The first is that ATP is required to form the phosphorylated intermediate—either because this intermediate takes part in the exchange, or because the process of phosphorylation is itself associated with the exchange. An alternative hypothesis is that, without any transfer of phosphate, ATP sitting on the pump sites does something—produces a configurational change perhaps—without which the shuttling of sodium cannot occur. The second hypothesis may sound somewhat contrived, but there is evidence for this kind of action of ATP in connexion with the binding of cardiac glycosides to the pump sites. In 1967 Matsui & Schwartz showed that the binding of tritiated digoxin to a membrane

TABLE 1. THE EFFECTS OF VARIOUS NUCLEOTIDES ON THE BINDING OF OUABAIN BY GHOSTS

(From Hoffman (1969). Reprinted with permission from *J. gen. Physiol.* **54**, 343s.)

| nucleotide added | mmol/l | molecules per ghost |
|---|---|---|
| ATP | 2.0 | 174 |
| | 0.01 | 152 |
| | 0.001 | 75 |
| ITP | 2.0 | 189 |
| | 0.01 | 160 |
| | 0.001 | 60 |
| | 0.0001 | 15 |
| CTP | 2.0 | 168 |
| | 0.01 | 161 |
| | 0.001 | 90 |
| ADP | 2.0 | 184 |
| | 0.01 | 144 |
| IDP | 0.01 | 13 |
| AMP | 2.0 | 7 |

Ghosts were incubated for 30 min at 37 °C before washing and counting in a medium which contained 40 mmol/l NaCl, 1.25 mmol/l $MgCl_2$, 0.25 mmol/l EDTA, 10 mmol/l tris buffer, $2.4 \times 10^{-7}$ mol/l tritiated ouabain, and the indicated concentrations of the different nucleotides.

preparation from calf heart was promoted by a combination of ATP, magnesium and sodium ions; and because this is the same combination as that which promotes phosphorylation, they suggested that phosphorylation was a prerequisite for glycoside binding. Later work, however, showed that other nucleoside triphosphates, which were hydrolysed very slowly or not at all by the pump, were as effective as ATP at promoting binding (Schwartz, Matsui & Laughter 1968; Albers, Koval & Siegel 1968; Hoffman 1969). It might be argued that if the cardiac glycoside traps the phosphorylated intermediate as soon as it is formed, even a very low rate of turnover would suffice to promote glycoside binding; but that explanation of the effects of inosine triphosphate (ITP) and cytidine triphosphate (CTP) is excluded by the experiment summarized in table 1. Even at nucleotide concentrations so low that the binding promoted by ATP was less than half maximal, ITP and CTP were still as effective as ATP. This experiment also disposes of the hypothesis that ITP and CTP act by forming ATP through some kind of transphosphorylation reaction. It is difficult to avoid the conclusion that nucleoside triphosphates can promote glycoside binding in some way which remains obscure but which does not involve phosphorylation.

Now let us return to the Na–Na exchange. To see whether ATP is needed to phosphorylate, or whether its action is more likely to be related to whatever it does in promoting glycoside binding, we have to determine whether ATP is required specifically or whether other nucleoside triphosphates can be substituted. To find out, resealed ghosts containing a variety of

nucleoside triphosphates were prepared from cells that had first been incubated without substrate for 24 h to deplete them of endogenous nucleotides. The ouabain-sensitive losses of $^{24}Na$ from these ghosts into a 10 mmol/l K medium, and into a K-free high-Na medium, gave a measure of the Na–K and Na–Na exchanges. The results of several experiments are summarized in figure 3 and it is clear that only the ghosts containing ATP showed appreciable Na–Na exchange. The specificity of the nucleotide requirement for promoting Na–Na exchange is therefore similar to that for promoting Na–K exchange, or for forming the phosphorylated intermediate, and quite different from that for promoting ouabain binding.

In the squid axon Na–Na exchange can be demonstrated only if the axon is partially poisoned (Baker *et al.* 1969), and there is a good deal of evidence, most recently from the work of De Weer (1968, 1970) in Baltimore, that Na–Na exchange in the axon occurs only when the ratio ATP/ADP is not too high. To determine whether this was a general feature of the pump it seemed worth while to see if the same kind of behaviour could be observed in red cells.

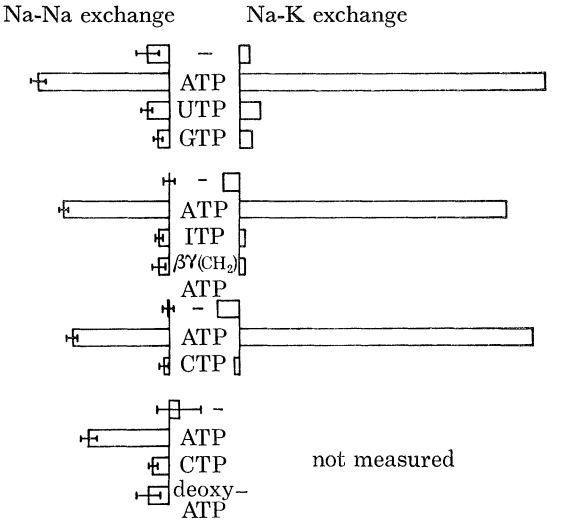


FIGURE 3. Nucleoside triphosphate requirements for Na–Na and Na–K exchange in resealed red cell ghosts.

It is easy to prepare resealed red cell ghosts containing different amounts of ATP and ADP, but the ghost volume is very small and the concentrations will soon change, through hydrolysis of ATP and also as a result of the activity of adenylate kinase. These difficulties can be partly overcome by incorporating into the cells an ATP-regenerating system consisting of creatine phosphate and creatine kinase. Ideally, one would like to maintain the ATP/ADP ratio at a predetermined level by incorporating large amounts of creatine and creatine phosphate in suitable proportions. Unfortunately, the Lohmann reaction is poised in such a way that, unless the ADP level is to be kept very low, rather high concentrations of creatine are necessary, and these concentrations seem to be toxic. Whether this toxicity is an effect of creatine itself or of a contaminant we do not know. But though it is not possible to buffer the ATP/ADP ratio entirely, it is possible, by varying the amounts of creatine phosphate and ATP in the lysing solutions, to prepare ghosts with ATP and ADP contents which are very different and which change relatively little during a 30 min incubation (see figure 4).

Figure 5 summarizes the results of two experiments in which resealed ghosts containing different amounts of ATP and ADP were allowed to lose $^{24}Na$ into a 10 mmol/l K medium and

a K-free high-Na medium in the presence and absence of ouabain. The ouabain-sensitive loss into the 10 mmol/l K medium gave a measure of Na–K exchange: the ouabain-sensitive loss into the K-free medium a measure of Na–Na exchange. The figure shows the relation between the magnitudes of the two exchanges and the nucleotide concentrations in the ghosts. It is clear that the two exchanges behaved quite differently. Na–K exchange varied roughly in parallel with the concentration of ATP and was little affected by ADP. The Na–Na exchange varied with the concentration of ADP, being relatively large when this concentration was high and almost absent when there was little ADP in the cells. These results point to a role for ADP in Na–Na exchange, though not in the exchange of sodium for potassium, and we must next consider what this role might be.

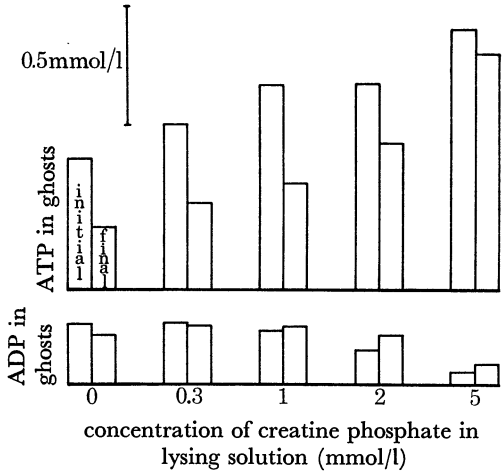


FIGURE 4. The use of creatine phosphate and creatine kinase to control ATP and ADP levels. All lysing solutions contained 1 mmol/l ATP; creatine kinase was present in those which contained creatine phosphate. After lysis, KCl was added to restore isotonicity, and the ghosts were incubated for 45 min to reseal them. They were then washed, and incubated for a further 30 min in a high-Na, K-free medium. The 'initial' and 'final' levels of the nucleotides shown in the diagram are the levels at the beginning and end of this 30 min period.

There are two obvious ways in which ADP might react with the pump system. It might act as a competitive inhibitor—competing with ATP—or it might reverse the reaction of which it is itself a product

$$\mathrm{ATP} + \mathrm{E} \rightleftharpoons \mathrm{E} \sim \mathrm{P} + \mathrm{ADP}.$$

Competitive inhibition can be ruled out straight away, since the action of a competitive inhibitor can always be simulated by an appropriate lowering of the concentration of substrate, and lowering the concentration of ATP does not promote Na–Na exchange. This leaves us with the hypothesis that ADP acts by reversing the reaction of which it is a product, and we must therefore ask why such a reversal should be necessary for Na–Na exchange. An attractive hypothesis is that the transphosphorylation is associated directly with the movement of sodium, so that a transfer of phosphate from ATP to E is associated with an outward movement of sodium, and a transfer from E ~ P to ADP is associated with an inward movement. This would explain why the backward and forward shuttling of sodium requires ADP as well as ATP, whereas only ATP is needed for the forward running of the pump (*see note added in proof*).

An alternative hypothesis is that ATP acts by keeping the concentration of E ~ P below a level at which it becomes inhibitory. Suppose, for example, that the translocation is actually done by E ~ P and that in moving inwards and outwards it has to occupy a particular site.

If when E ~ P has moved outwards, carrying sodium to the outer side of the membrane, the site is occupied by a further molecule of E ~ P coming from inside, then the outside molecule will be unable to get back, the inside molecule will be unable to move outwards, and the exchange will be blocked. A possible objection to this hypothesis is that it requires that more

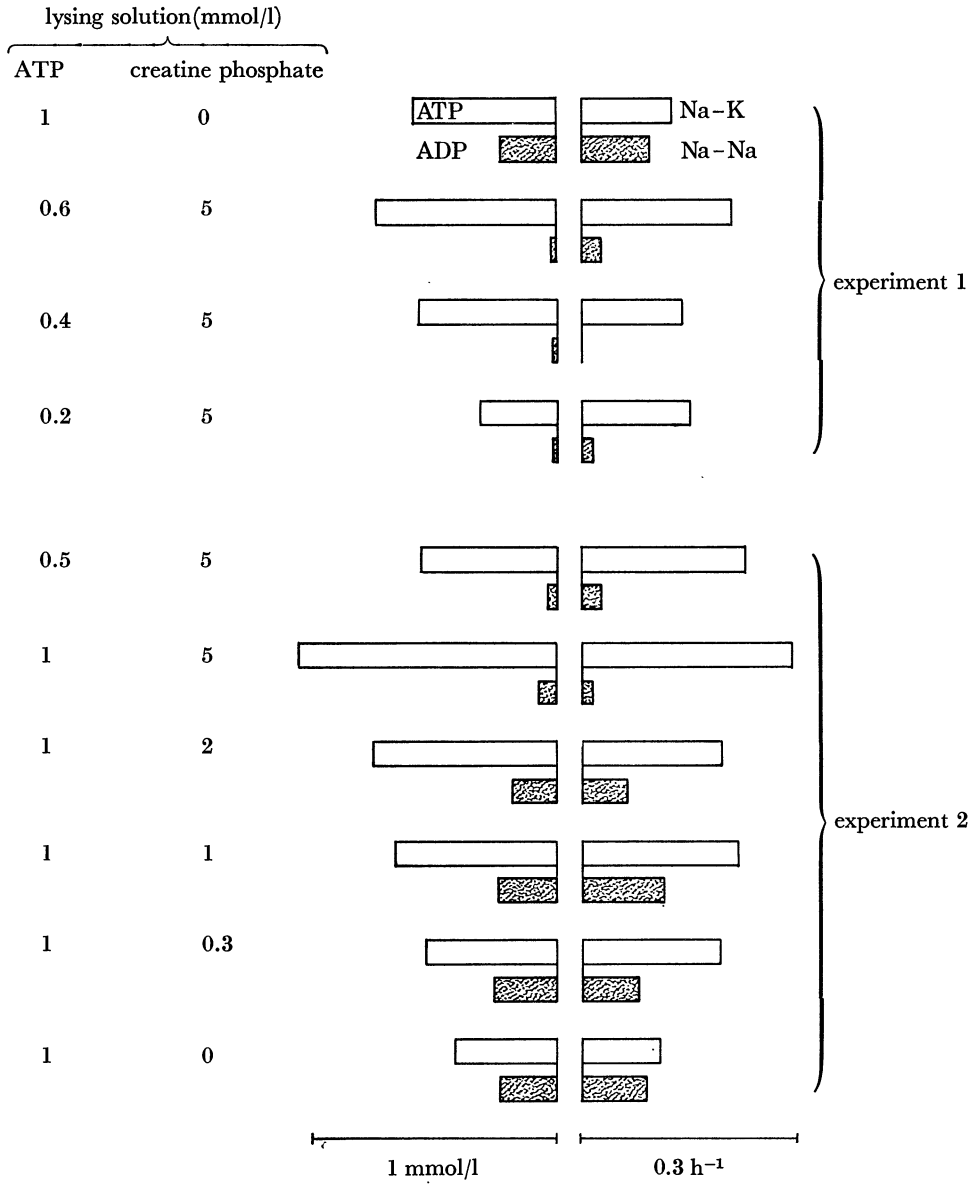


FIGURE 5. The effects of ATP on the Na–K (unshaded), and of ADP on the Na–Na (shaded) exchanges. The columns on the right show the magnitudes of the ouabain-sensitive Na effluxes when the resealed ghosts were incubated in 10 mmol/l K or K-free high-Na media. In experiment 1 the columns on the left show the nucleotide levels at the beginning of the 30 min incubation period during which the fluxes were measured. In experiment 2 each left-hand column shows the mean of the initial and final nucleotide levels.

than one molecule of E ~ P must be able to exist at each site; and this is quite uncertain (Albers *et al.* 1968). But, in any event, a slight modification of the hypothesis makes it tenable even if only one molecule of E ~ P can exist. Instead of supposing that E ~ P does the shuttling, let us suppose that E ~ P is used to form a pyrophosphate bond which behaves as

suggested in the recent ingenious theory of Wang (1970) illustrated by figure 6. Wang imagines a channel, with phosphate groups lining it, extending through the membrane, and he assumes that energy from ATP can be used to form a pyrophosphate bond which is able to migrate freely backwards and forwards along the channel carrying sodium with it. He postulates that the pyrophosphate bonds are hydrolysed at the outer surface of the membrane by a potassium-activated phosphatase, and that it is the formation of these bonds at the inner surface and their destruction at the outer that accounts for the active outward transport of sodium. In the absence of external potassium Wang's model would allow a Na–Na exchange, and because of the single file effect this exchange would presumably be maximal in any channel when the channel contained only one pyrophosphate bond. Excessive formation of E ~ P could therefore block

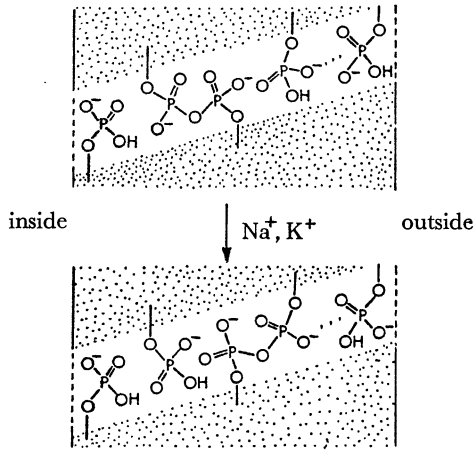


FIGURE 6. J. H. Wang's hypothetical model for the active transport of $Na^+$ driven by the outward diffusion of the pyrophosphate linkage. Reprinted with permission from the *Proceedings of the National Academy of Sciences* **67**, 59 (1970).

Na–Na exchange by forming further bonds per channel, and this is true even if not more than one molecule of E ~ P can exist at a time. Unfortunately, Wang's hypothesis is less satisfactory at explaining the normal functioning of the pump, since it does not account for the coupled potassium movement when this movement cannot be attributed to the electrical effect of the sodium transport.

The obvious way to distinguish between (i) the hypothesis that ADP is necessary because the inward movement of sodium is directly associated with a transfer of phosphate from E ~ P to ADP, and (ii) the hypothesis that ADP is necessary to hold down the concentration of E ~ P or to limit the fraction of time during which it exists, is to compare ATP–ADP exchange with Na–Na exchange. Unfortunately, this is technically difficult, because to measure Na–Na exchange it is necessary to work with intact cells, and in intact cells the adenylate kinase makes measurements of ATP–ADP exchange useless. The interpretation of these measurements may be further complicated by compartmentation of the nucleotides (Parker & Hoffman 1967).

## POTASSIUM ENTRY AND DEPHOSPHORYLATION

Let us now turn to the potassium side of the pump. The catalytic effect of potassium ions on the hydrolysis of the phosphorylated intermediate suggests that these ions are involved in a dephosphorylation step, and this is also suggested by two other lines of evidence.

First, membrane fragments with (Na + K)-dependent ATPase activity are able to hydrolyse substrates like acetyl phosphate, carbamyl phosphate and *p*-nitrophenyl phosphate; and, like the hydrolysis of ATP, the hydrolysis of these substrates requires potassium ions and is inhibited by cardiac glycosides (for references, see Glynn 1968). There has been a good deal of argument about whether this phosphatase activity is related to the pump, but it now seems clear that it is. The difference in the apparent $K_m$ for $K^+$ activation of the ATPase activity and of the phosphatase activity is not evidence that two separate enzymes are responsible, since the $K_m$ for $K^+$ activation of the phosphatase is different for different substrates (Yoshida, Izumi & Nagai 1966; Bader & Sen 1966). Recent evidence that the phosphatase activity is related to the pump comes

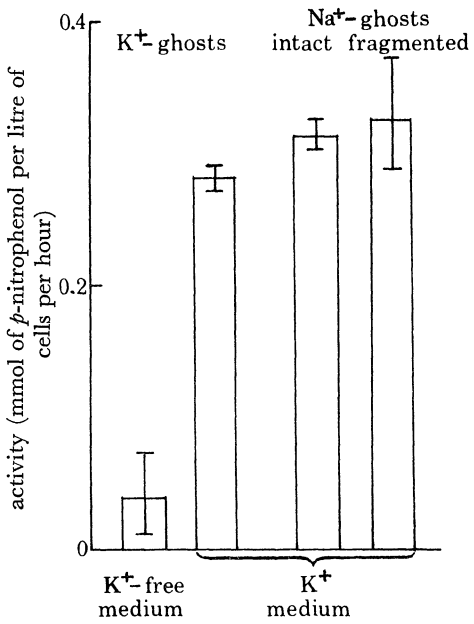


FIGURE 7. Ouabain-sensitive hydrolysis of *p*-nitrophenyl phosphate in reconstituted ghosts of red cells and in ghosts fragmented by freezing and thawing. (From Rega *et al.* (1970). Reprinted with permission from *Science, N.Y.* **167**, 55.)

from experiments on the effects of sodium and nucleotides on phosphatase activity, which will be discussed later, and from experiments on resealed red cell ghosts (Garrahan, Pouchan & Rega 1969; Rega, Pouchan & Garrahan 1970; Rega, Garrahan & Pouchan 1970). These experiments show that for phosphatase activity to be detected, the substrate and magnesium ions must be at the inner surface of the membrane but the potassium ions must be at the outer surface (see figure 7). A crucial question is whether the hydrolysis of the phosphatase substrates is accompanied by an inward movement of potassium ions, but technical difficulties have so far made this apparently simple question impossible to answer. Askari & Rao (1969) have recently claimed that the potassium-activated hydrolysis of *p*-nitrophenyl phosphate can generate an outward movement of sodium ions from resealed red cell ghosts. This is surprising and, if confirmed, is obviously important.

Quite separate evidence linking potassium entry with dephosphorylation comes from experiments suggesting that the reverse process—an outward movement of potassium through the pump—requires inorganic phosphate. In red cells—and possibly in other cells, but nobody has looked—part of the downhill outward movement of potassium is inhibited by ouabain.

When this outward movement of potassium is into a medium lacking potassium but rich in sodium, it appears to be associated with the backward running of the entire pump cycle and it is accompanied by the incorporation of inorganic phosphate into ATP. When the medium contains K however, the whole cycle does not run backwards but, provided inorganic phosphate is present inside the cells, an exchange of internal for external potassium ions takes place superimposed on the normal forward running of the pump (Glynn, Lew & Lüthi 1970; Glynn & Lew 1970). One may imagine that the dephosphorylation accompanying potassium entry is usually followed by rephosphorylation at the expense of ATP, and the subsequent expulsion of sodium, but that just occasionally the rephosphorylation is at the expense of inorganic phosphate, and is associated with an outward movement of potassium ions.

TABLE 2. THE EFFECT OF NUCLEOTIDE DEPLETION ON OUABAIN-SENSITIVE K EFFLUX

| initial ATP content of cells ($\mu$mol/l cells) | medium | fraction of K lost in 1 h (%) control | ouabain | difference |
|---|---|---|---|---|
| 438 | K-free | 3.19 ± 0.05 | 2.87 ± 0.03 | 0.315 ± 0.059 |
| | 10 mmol/l K | 3.64 ± 0.04 | 3.24 ± 0.04 | 0.398 ± 0.057 |
| < 2 | K-free | 2.21 ± 0.02 | 2.21 ± 0.02 | 0.002 ± 0.025 |
| | 10 mmol/l K | 2.38 ± 0.03 | 2.34 ± 0.03 | 0.041 ± 0.045 |

Table 3. EVIDENCE THAT THE LACK OF EFFECT OF OUABAIN ON K EFFLUX FROM DEPLETED CELLS WAS NOT THE RESULT OF A FAILURE TO BIND OUABAIN

| conditions of incubation of intact depleted cells | ATP hydrolysed by washed ghosts (mmol/(l orig. cells).h) control | ouabain | difference |
|---|---|---|---|
| 10 mmol/l K | 0.546 | 0.147 | 0.399 |
| K-free | 0.588 | 0.127 | 0.461 |
| 10 mmol/l K + ouabain | 0.163 | 0.095 | 0.068 |
| K-free + ouabain | 0.202 | 0.130 | 0.071 |

All this leads us to think of the pump as a reversible phosphokinase associated with sodium movement, coupled to a reversible phosphatase associated with the movement of potassium; but two recent pieces of evidence suggest that the situation is more complicated.

First, Yoshida, Nagai, Ohashi & Nakagawa (1969), working with a brain membrane preparation, and Garrahan, Pouchan & Rega (1970), working with red cell membranes, have looked at the effects of sodium and of ATP on the potassium activated hydrolysis of *p*-nitrophenyl phosphate (*p*NPP). In both preparations, sodium alone inhibits, probably by competing with potassium ions, and ATP alone inhibits, probably by competing with the substrate. Surprisingly, however, ATP and sodium together stimulate *p*-nitrophenyl phosphatase activity by increasing the apparent affinity of the phosphatase for potassium ions. In the experiments of Yoshida *et al.*, inosine triphosphate (ITP) and ADP were only a little less effective than ATP. Since the membrane preparation they used is reported to hydrolyse ITP and ADP only very slowly, and ADP produced its effect on the affinity of the phosphatase without any delay, it is unlikely that the effect of the nucleotide plus sodium involves the formation of a phosphorylated intermediate. The interpretation of these results is not clear, but they suggest that nucleotides may be involved in the potassium part of the pump cycle.


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Independent evidence for this comes from an experiment showing that the ouabain-sensitive efflux of potassium that occurs under normal conditions was absent or greatly reduced in cells completely lacking ATP and ADP. A difficulty with experiments of this kind is that the prolonged incubation normally necessary to deplete the cells of their energy stores increases the potassium leak sufficiently to prevent the detection of the small ouabain-sensitive efflux. To circumvent this difficulty, cells that had been incubated for 3 h without glucose were incubated for a further 2 h in a 150 mmol/l phosphate medium containing inosine (5 mmol/l) and iodoacetamide (5 mmol/l). Inosine enters the cells and is phosphorolytically split to hypoxanthine and ribose phosphate which then undergoes a complicated series of reactions, the net effect of which is to consume ATP and form triose phosphate. ATP and ADP are reduced to extremely low levels in about 30 min (V. L. Lew, unpublished work). Table 2 shows the ouabain-sensitive efflux of potassium from cells depleted in this way and from cells from the same batch that had not been incubated with inosine and iodoacetamide. Efflux into a K-free medium is associated with a reversal of the whole cycle and it is not surprising that it was absent in the depleted cells. What is surprising is the absence of a significant ouabain-sensitive potassium efflux from the depleted cells incubated in a 10 mmol/l K medium. This implies that either ATP or ADP is somehow involved in the K:K exchange. An alternative explanation of these results—that the glycoside was unable to act in the absence of nucleotide—can be excluded because when the depleted cells that has been exposed to ouabain were washed and lysed, the (Na + K)-dependent ATPase activity of the ghosts was found to be almost completely inhibited (table 3).

Why K–K exchange should require the presence of nucleotide is not at all clear. It would be helpful to know whether ATP specifically is required or whether other nucleotides can act instead, but since there is no way of getting nucleotide into depleted cells it is not going to be easy to find out. All that can be said at this stage is that it is likely to be misleading to think of the two halves of the pump cycle too independently.

This work was supported in part by USPHS grants HE 09906 and AM 05644.

[*Note added in proof*, 19 May 1971.] Whether or not the movement of sodium ions is associated with the transfer of phosphate between ATP and E ~ P, Na–Na exchange must involve a step subsequent to the transphosphorylation since oligomycin stimulates ATP–ADP exchange (Blostein 1970) but inhibits Na–Na exchange (Garrahan & Glynn 1967*c*).

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