Bioenergetics (1973) **4**, 1–30

The Relationship of the ($Na^+ + K^+$)-Activated Enzyme System to Transport of Sodium and Potassium Across the Cell Membrane

J. C. Skou

Institute of Physiology, University of Aarhus, 8000 Aarhus C, Denmark

I. *Introduction*

The energy-requiring, active, transport of sodium out and potassium into the cell seems to be due to the membrane bound (Na + K)-activated enzyme system.[1–6] This system seems also to be involved in the Na:Na and K:K exchange which under certain conditions takes place across the cell membrane.[4,7] It has been named an enzyme system [1] since it not only catalyzes the hydrolysis of ATP but takes part in the reaction in the sense that the hydrolysis of ATP via this system seems to be translated into the vectorial movement of the cations against the electrochemical gradients, i.e. it seems to be identical with the transport system.

A detailed knowledge of the system and the way it functions may lead to an understanding as to how sodium and potassium are transported across the cell membrane. This would include answers to at least three questions:

(1) What is the molecular structure of the system.
(2) How is the relationship between the effect of sodium, potassium, magnesium, and ATP on the system and what is the sequence of steps in the reaction which leads to the hydrolysis of ATP.
(3) What happens on the molecular level when the system reacts with sodium, potassium, magnesium, and ATP.

The questions cannot be answered at present. There is, however, a number of observations on the system which makes it possible to discuss the sequence of some of the steps in the reaction and to discuss some of the problems to solve to get more insight in the transport mechanism.

II. *Main Characteristics of the System*

The substrate for the system is ATP, which is hydrolyzed to ADP and Pi.[1] The system requires a combined effect of sodium and potassium for

activation,[8] and in the intact cell the effect of sodium is from the inside and of potassium from the outside.[9–12] The number of cations necessary for activation is unknown, but if the activator sites are identical with the carrier sites for the transport of the cations, there must be at least three sodium ions and two potassium ions necessary.[4] The system has thus two sets of sites, one set located on the inside of the membrane (the i-sites), on which sodium is necessary for activation, and another set located on the outside (the o-sites), on which potassium is necessary for activation. There is a competition between sodium and potassium both for the i- and for the o-sites.[1]

With the isolated system in a test tube it is not possible to establish an asymmetric situation with a sodium medium in contact with the i-sites, and a potassium medium with the o-sites; both sets of sites are in

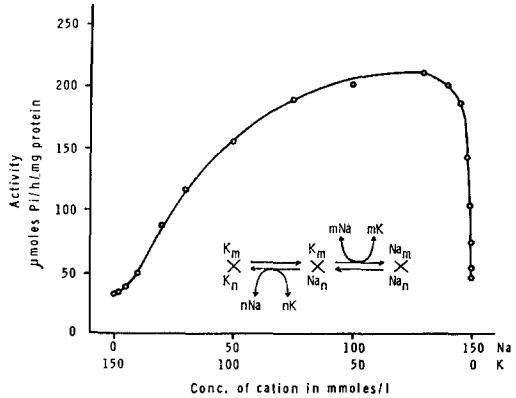


Figure 1. The effect of sodium plus potassium on the activity of the $(Na^+ + K^+)$-activated enzyme system, Mg^{2+} 6, ATP 3 mM, pH 7·4, 37°C. The enzyme was prepared from ox brain.[74]

contact with the same sodium-potassium medium. The ratio between the concentrations of sodium and potassium necessary to give half maximum saturation of the i-sites differs, however, so much from the ratio necessary to give half maximum saturation of the o-sites that it is possible with certain concentrations of sodium and potassium in the test tube to have a situation where a major part of the system is on the active K_m^o/Na_n^i form, Fig. 1. The ratios between sodium and potassium for half maximum activation read from the curve in Fig. 1 is about 1:3–4 for the ascending part of the curve (the sodium-activating sites, the i-sites), and about 100:1 for the descending part (the potassium-activating sites, the o-sites). From these values it can be calculated that the activity of the system with concentrations of sodium and potassium which give maximum activity, 130 and 20 mM, respectively, is about 80–85% of the activity which could be obtained if the i-sites were in contact with a sodium solution and the o-sites with a potassium

solution. The asymmetry of the curve reflects how much the apparent sodium/potassium affinity ratio for the i-units differs from the apparent potassium/sodium affinity ratio for the o-units.

It is furthermore a characteristic property of the system that it is inhibited by cardiac glycosides.[1–6]

III. *Preparation and Purity of the Enzyme System*

The enzyme system is located in a cell membrane and preparations of the enzyme system consist of membrane pieces isolated by a differential centrifugation of a tissue homogenate. Membrane pieces which contain the system are found in all the sediments after a differential centrifugation. In the heavier sediments the (Na + K)-activated activity is manifest, while in the lighter sediments—heavy and light microsomes—part of the activity is latent. The explanation seems to be that the small membrane pieces form vesicles with no access for either MgNaATP, or potassium to the one side of the membrane.[13–15]

The latent activity can become manifest by treatment of the membrane vesicles with detergents like DOC,[16] high concentrations of salt like sodium iodide,[17] and by freezing.[14] Up to a certain concentration both salt and detergents will increase the activity while they again decrease the activity when used in higher concentrations. The effect of the detergents are highly dependent on temperature, time, concentration of protein in the solution and for the ionized detergents of pH[13]. This means that for optimum activation the treatment has to be done under control of all these parameters.

There are two effects of the activation. One is that the latent activity becomes manifest, apparently due to an opening of the vesicles;[13–15] this gives an increase in specific activity. The other is a release of inactive protein which goes into solution.[13,14] It may be protein which has been trapped inside the vesicles and is released when the vesicles are opened and/or protein released from the membrane *per se*. It amounts to 40–50% of the protein in the preparation prior to a treatment with the detergents. The membrane particles which contain the enzyme activity can be separated from the released dissolved protein by centrifugation, and this gives a further increase in the specific activity.

The membranes always contain a magnesium activated ATPase besides the (Na + K)-activated. The ratio between the two activities varies from tissue to tissue and also depends on the preparative procedure. The magnesium-activated ATPase is more labile than the (Na + K)-activated towards SH-blocking agents[18] and treatment with high concentrations of salt.[17] Most of this activity disappears in the procedures where high concentrations of salt are used to activate

the system.[18] High concentrations of sodium iodide can also be used to decrease the magnesium activity in the preparations activated by detergents.[19]

Part of the magnesium-activated ATPase may stem from mitochondrial contamination of the membranes. It is unknown whether the other part of the magnesium-activated ATPase has any relation to the $(Na^+ + K^+)$-activated ATPase.

A further purification of the $(Na^+ + K^+)$-activated enzyme system can be obtained by density gradient centrifugation of the detergent activated membrane preparations,[20] by ammonium sulphate fractionation,[21] or gel filtration[22,23] of detergent solubilized preparations. The specific activity which can be obtained by the density gradient centrifugation depends on the tissue used as starting material. This may mean that membranes from different tissues has a different density of enzyme sites. The outer medulla from rabbit kidney is the tissue which has so far given preparations with the highest specific activity, namely about 1500 μM Pi/mg protein/hour.[20] In this preparation the enzyme system is still bound to membrane pieces, and is estimated to be maximally 49% pure.

The number of enzyme units/mg protein of enzyme preparations has been determined either by measuring the sodium-dependent incorporation of P^{32} from ATP^{32},[21,22,24,25] the binding of ATP^{32},[26–28] or the binding of labelled g-strophanthin.[25,28–31] In experiments with enzyme preparations from different tissues where the number of binding sites on the same enzyme preparation has been determined by P^{32} labelling and cardiac glycoside binding,[25] by P^{32} labelling and ATP binding,[27] and by ATP and cardiac glycoside binding,[28] the correlation, with one exception,[25] has been close to 1·0. Assuming one binding site per enzyme unit, the molecular activity varies from about 3000 to about 15,000 molecules Pi/enzyme unit/minute for preparations from different tissues. This may mean that the molecular activity of preparations from different tissues varies, or it may reflect that it is difficult to obtain reliable values for the site numbers; there are problems with background labelling both with labelling by P^{32}, binding of ATP^{32}, and of labelled cardiac glycosides. It must also be emphasized that the molecular activity is calculated from the maximum activity of the system which can be obtained in the test tube, and this is as discussed in the previous section not the maximum activity of the system.

Molecular weight determinations by radiation inactivation have given values of the order of 500,000[32] and 250,000[33].

On a polyacrylamide gel the partly purified preparations dissolved in sodium dodecyl sulfate-mercaptoethanol give two major bands, one with a molecular weight of 94,000[21]–90,000[22] and another with a molecular weight of about 53,000.[22] The sodium-dependent P^{32} labelling from ATP^{32} is found in the 90,000–94,000 molecular weight

band,[21,34] which suggests that a polypeptide of this molecular weight is part of the system.

IV. *Lipids for Activation*

Extraction of loosely bound lipids, cholesterol and part of the phospholipids, has no effect on the activity of the $(Na^+ + K^+)$-activated enzyme system,[35] while extraction of the more firmly bound phospholipids by polar solvents inactivates the system.[35–36] The inactivation may be due to the removal of the lipids or to a denaturing effect of the solvents.

Treatment with crude preparations of phospholipase A and C[36–40] leads to an inactivation; so does treatment with a highly purified preparation of phospholipase A.[35] In the phospholipase-treated preparations which have been partly inactivated, the addition of lecithin[36] or asolectin,[40] a commercial soybean extract, gives a certain, but low reactivation, while no reactivation was seen by addition of lipids to completely inactivated preparations.

In enzyme preparations in which the latent enzyme activity has not been uncovered by treatment with detergents or high concentrations of salt, the addition of phosphotidylserine gives a certain but low increase in activity.[41,42] It seems most likely that this is due to a detergent-like effect of the phospholipids.[35]

DOC-solubilized inactivated enzyme preparations can to some extent be reactivated by addition of lipids.[23,42–47] Tanaka and Sakamuto[47] found that reactivation could be obtained by acidic phospholipids such as phosphatidic acid, phosphatidylinositol and phosphatidylserine, whereas neutral lipids as lecithin and phosphatidylethanolamine were inactive. Besides the acidic phospholipids, mono- and diacyl phosphates could activate, and they concluded that the essential structure needed for activation is a phosphate group plus one or two fatty acyl residues. Wheeler and Whittam[42] found that crude commercial samples of acidic phospholipids reactivated, but after partial purification by chromatography definite activation was only shown with the components which migrated like phosphatidylserine. It is not possible from the experiments on the reactivation of the DOC-solubilized inactivated preparations to exclude that the lipid reactivation is due to a removal of the detergents from the enzyme by the lipids and not to an effect of the lipids per se on the enzyme. On the other hand, the apparent requirement for specific lipids may suggest that it is due to an effect of the lipids.

An interesting observation concerning requirement for lipids for the transport system is that the amount of sulfatides in preparations from salt glands from duck increases parallel with the $(Na^+ + K^+)$-ATPase during a salt load,[48] while other lipids increased less.

V. *Intermediary Steps in the Reaction*

The system hydrolyzes ATP to ADP and Pi in the presence of magnesium, sodium, and potassium. ATP forms a complex with magnesium, which means that the solution contains a mixture of Mg^{2+}, MgATP, and free ATP (ATP_f). To understand the sequence of the reaction which leads to the hydrolysis of ATP it is necessary to know the relationship between the effect of all these components on the system. At present, our knowledge about this is sparse, partly because there is no way to vary Mg^{2+}, MgATP, and independently, partly because there are few ways to investigate each of the steps in the reaction independently.

A. *Without Magnesium*

At 0°C, the system binds ATP with a high affinity with no magnesium, sodium, or potassium in the medium;[26,27] the dissociation constant is about 0·2 μM. Sodium has no effect on the affinity for ATP, while potassium decreases the affinity.[26,27]

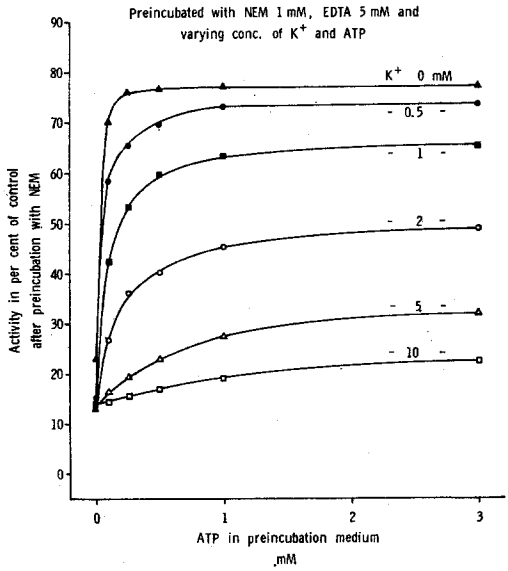


Figure 2. The effect of potassium and ATP on the inhibition of the $(Na^+ + K^+)$-activated enzyme by 1 mM n-ethylmaleimide (NEM). The enzyme was preincubated with 1 mM NEM 5mM EDTA, and the concentration of potassium and ATP shown on the figure in 30 mM Tris HCl buffer, pH 7.4 at 37°C for 30 min. After preincubation, the activity of the preparation was tested by transferring 0·1 ml of the preincubation medium to 1 ml of test solution with a final concentration of 3 mM magnesium, 3 mM ATP, 120 mM sodium, 30 mM potassium, 1 mM β-mercaptoethanol, 30 mM Tris HCl, pH 7·4, 37°C. Control was enzyme preincubated without NEM (unpublished).

The system has, as discussed above, two sets of sites, the i-sites and the o-sites with affinities for cations. The binding experiments do not tell whether the effect of potassium on the affinity for ATP is on the i- or on the o-sites. Information about this may come from experiments where the inhibitory effect of n-ethyl maleimide (NEM) has been used as a tool to test the effect of sodium and potassium on the effect of ATP on the system. ATP protects against the inhibitory effect of NEM. Potassium and sodium decreases the protective effect of ATP, but in a different way. In agreement with the results from the binding studies,

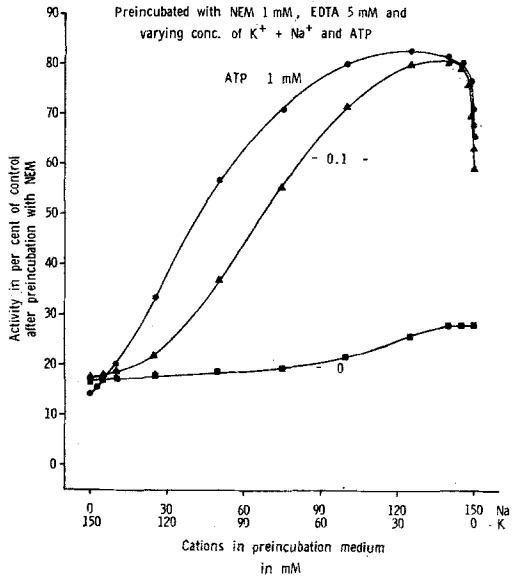
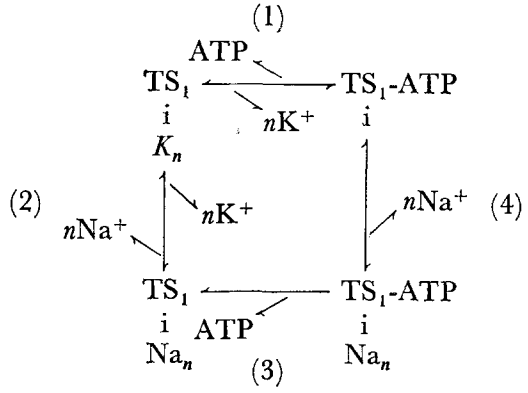


Figure 3. The effect of sodium, potassium, and ATP on the inhibition of the $(Na^+ + K^+)$-activated enzyme system by 1 mM NEM. The enzyme was preincubated with 1 mM NEM, 5 mM EDTA, and the concentration of sodium, potassium, and ATP shown on the figure in 30 mM Tris HCl buffer, pH 7·4 at 37°C for 30 min. After preincubation, the activity of the preparation was tested as described in Fig. 2 (unpublished).

potassium decreases the protection by decreasing the apparent affinity for ATP, Fig. 2, while sodium has no effect on the apparent affinity (not shown). Both potassium and sodium decreases the maximum level of protection which can be obtained by ATP, but the effect of potassium is much more pronounced than that of sodium, see Fig. 3.

Figure 3 shows that the inhibitory effect of potassium can be titrated away by sodium, and the inhibitory effect of sodium can be titrated away by potassium. A comparison between Figs. 1 and 3 (the left hand part of the curves) shows that the concentration of sodium necessary to give half maximum removal of the inhibitory effect of potassium with 1 mM ATP (Fig. 3) is of the same size as the concentration of sodium

to give half maximum activation of the hydrolysis (Fig. 1). This suggests that the inhibitory effect of potassium on the protection by ATP against NEM is due to an effect of potassium on the sodium-activating site of the system, the i-sites. Figure 3 furthermore shows that ATP increases the apparent affinity of this site for sodium relative to potassium. The simplest way to explain the observations on the effect of sodium and potassium on the binding of ATP and on the protection of ATP against the effect of NEM is that the system with the i-sites on the potassium form does not react with ATP_f while without potassium, or with the i-sites on the sodium form, it does. (TS for transport system, i for the sodium-activating sites on the inside of the membrane, *n* is a number).



B. *With Magnesium*

The binding experiments and the experiments on the inhibition by NEM show that magnesium is not necessary for the reaction with ATP. Magnesium is, however, necessary for the hydrolysis of ATP. What is then the effect of magnesium? Which of the components, Mg^{2+}, MgATP, and ATP_f does the system react with when the medium contains magnesium plus ATP, and what is the sequence?

As a basis for the discussion of this problem it is convenient to use the following simple scheme for the overall hydrolysis of ATP by the enzyme system with magnesium, ATP, sodium, and potassium in the medium:

$$E + ATP \rightleftharpoons E\text{-}ATP \tag{5}$$

$$E\text{-}ATP \overset{Na^+,\ Mg^{2+}}{\rightleftharpoons} E \sim P + ADP \tag{6}$$

$$E \sim P + H_2O \overset{K^+}{\rightleftharpoons} E + Pi \tag{7}$$

$$E + ATP + H_2O \overset{Mg^{2+},\ Na^+,\ K^+}{\rightleftharpoons} E + ADP + Pi \tag{8}$$

It is based on the observation that the reaction with magnesium, ATP, and sodium leads to a phosphorylation-dephosphorylation of

the system. The rate of phosphorylation is high, while that of dephosphorylation is low. When potassium is added to a prephosphorylated preparation, the rate of dephosphorylation is increased.[3,49]

As seen from the scheme, the system can accomplish a magnesium–sodium-dependent ATP–ADP exchange. It was observed by Fahn *et al.*[50] that the magnesium requirement for the exchange reaction is much lower than for the $(Na^+ + K^+)$-dependent hydrolysis of ATP. This led to the suggestion that two magnesium molecules and two phospho-enzymes are involved in the reaction. One magnesium molecule for which the system has a high affinity and which is necessary for the formation of the phosphorylated intermediate which takes part in the exchange reaction, (9) and (10) in the following scheme.[50] Another magnesium molecule for which the affinity is an order of magnitude lower and which is necessary for the transformation of the phosphorylated intermediate into a form which can react with potassium and be dephosphorylated, (11), (12) and (13).

$$E + Mg^{2+} \leftrightharpoons Mg\text{-}E \tag{9}$$

$$Mg\text{-}E + ATP \overset{Na^+}{\leftrightharpoons} Mg\text{-}E \sim P + ADP \tag{10}$$

$$Mg\text{-}E \sim P + Mg^{2+} \leftrightharpoons Mg\text{-}E \sim P\text{-}Mg \tag{11}$$

$$Mg\text{-}E \sim P\text{-}Mg \overset{(Na^+\ ?)}{\leftrightharpoons} Mg\text{-}E\text{-}P\text{-}Mg \tag{12}$$

$$Mg\text{-}E\text{-}P\text{-}Mg + H_2O \overset{K^+}{\leftrightharpoons} Mg\text{-}E + Pi + Mg^{2+} \tag{13}$$

C. *With Magnesium, ATP, and Sodium*

Evidence for the existence of two different phosphorylated intermediates has been given by Post *et al.*[51] In experiments at 0°C they were able to show that the phosphorylated intermediates formed with a concentration of magnesium which was low and high, respectively, relative to the concentration of ATP, differed in their reactivity towards ADP, potassium, and g-strophanthin. When formed with a very low concentration of magnesium, the addition of ADP led to an increased rate of dephosphorylation, while potassium had a low or no effect. When formed with a higher magnesium concentration, the addition of potassium led to an increased rate of dephosphorylation, while ADP had a low or no effect. The ADP-sensitive phospho-enzyme did not react with g-strophanthin, while the potassium-sensitive did. This difference in sensitivity towards g-strophanthin is in disagreement with the observation that g-strophanthin inhibits the exchange reaction,[50] but apart from this, which may be due to differences in experimental conditions, the two phospho-enzymes behave as predicted from the scheme by Fahn *et al.*[50] According to this scheme, it is the native enzyme which has the high affinity for magnesium (9), and the

phospho-enzyme (11), which has the low affinity for a second magnesium which is necessary for the transformation of E ~ P into E–P.

Results from experiments with g-strophanthin seem to support the view that there is a shift in the requirement for magnesium when ATP is hydrolyzed.[52] The experiments suggest, however, that the shift in the requirement for magnesium is due to a different way of interaction between sodium and magnesium with and without ATP.

Without ATP, sodium was found to decrease the apparent affinity for Mg^{2+}, while with ATP this effect disappears. Without sodium, free ATP inhibits the effect of magnesium plus ATP on the reaction by g-strophanthin. With sodium, the inhibitory effect of free ATP disappears. In other words, ATP seems to eliminate an antagonism between sodium and magnesium, while sodium eliminates an inhibitory effect of free ATP. The experiments suggest that with sodium in the medium it is the sodium-ATP form of the system which reacts with magnesium with a very high affinity. The elimination of the inhibitory effect of free ATP by sodium may either mean that the system on the sodium-ATP form has a magnesium site with a very high affinity for Mg^{2+} or that the sodium-ATP form does not require Mg^{2+}, but MgATP for the reaction with g-strophanthin.

The ADP-sensitive phospho-enzyme is formed under conditions where the concentration of magnesium is very low relative to the ATP concentration which suggests that free ATP does neither inhibit the sodium-dependent formation of the ADP-sensitive phospho-enzyme.

Considering this, it seems most likely that the sodium elimination of the inhibitory effect of free ATP means that Mg^{2+} at a magnesium site is not necessary for the reaction with g-strophanthin and for the formation of the ADP-sensitive phospho-enzyme, but MgATP. The sodium form of the system may react with MgATP with a high affinity; or, as the system binds ATP without magnesium, it may be that ATP bound to the sodium form of the system complexes Mg^{2+} with a high affinity, higher than for ATP in solution.

$$Na_n\text{-}TS_1 + MgATP \rightleftharpoons Na_n\text{-}TS_1\text{-}MgATP \qquad (14)$$

or

$$Na_n\text{-}TS_1\text{-}ATP + Mg^{2+} \rightleftharpoons Na_n\text{-}TS_1\text{-}MgATP \qquad (15)$$

On the Na_n-TS_1-MgATP form the system has catalytic activity, and the reaction leads to the formation of the ADP-sensitive phospho-enzyme.

$$Na_n\text{-}TS_1\text{-}MgATP \rightleftharpoons Na_n\text{-}TS_1 \sim P + Mg^{2+} + ADP \qquad (16)$$

A higher magnesium concentration relative to the ATP concentration is necessary for the formation of the potassium sensitive phospho-enzyme. Experiments on the reaction of the system with pNPP as substrate suggest that there is a site on the system which reacts with

Mg^{2+} independent of substrate.[53] It seems therefore likely that the formation of the potassium-sensitive phospho-enzyme requires a reaction of the system with Mg^{2+} at a magnesium site besides the reaction with MgATP;

$$Na_n\text{-}TS_1\text{-}MgATP + Mg^{2+} \leftrightharpoons Mg\text{-}Na_n\text{-}TS_1\text{-}MgATP \qquad (17)$$

The phosphate in the potassium-sensitive phospho-enzyme seems to be bound to the same group on the system as the phosphate in the ADP-sensitive phospho-enzyme;[51] this seems to be an acyl phosphate,[54–56] which means that it is bound in a high energy bond in both phospho-enzymes. The different sensitivity towards ADP and potassium of the two phospho-enzymes can therefore not be explained by a different way of phosphorylation—they must differ in some other way. The exchange reaction, i.e. the formation of the ADP-sensitive phospho-enzyme is insensitive to oligomycin, while the formation of the potassium-sensitive phospho-enzyme is sensitive to oligomycin.[57,58] The formation of the oligomycin-sensitive phospho-enzyme is more sensitive to a decrease in temperature than the formation of the ADP-sensitive.[57,58] NEM which blocks the hydrolysis of ATP with sodium plus potassium in the medium increases the exchange reaction, i.e. it apparently blocks the step which leads to the formation of the potassium-sensitive but not the ADP-sensitive phospho-enzyme.[59] The effects of temperature and of NEM on the formation of the potassium-sensitive phospho-enzyme may suggest that this step involves a change in conformation of the system, TS_1 to TS_2 (cf. refs. 51, 58), and that it is this difference in conformation that gives a different sensitivity of the acyl phosphate towards ADP and towards potassium.

$$Mg\text{-}Na_n\text{-}TS_1\text{-}MgATP \leftrightharpoons Mg\text{-}Na_n\text{-}TS_2 \sim P + ADP \qquad (18)$$

In the scheme given by Fahn *et al.*,[50] and by Post *et al.*,[51] the ADP-sensitive and the potassium-sensitive phospho-enzyme represents two consecutive steps in the reaction. Another possibility is, as shown above, that either the one or the other is formed dependent on the magnesium concentration.

As mentioned above, it is apparently the same group on the system which is phosphorylated in the ADP-sensitive and in the potassium-sensitive phospho-enzyme.[51] The phosphate seems to be bound as an acyl phosphate,[54–56] which means in a bond which has normally a free energy of hydrolysis, which is of the same size as for the hydrolysis of the γ–β phosphate bond in ATP.

The phosphorylation of the system is, however, not specific for the reaction with ATP. ITP,[49,60] AcP,[61–63] pNPP,[64] and Pi,[65,66] can also phosphorylate the system. The phosphorylation from ITP and AcP is dependent on sodium as is the phosphorylation from ATP (see, however, refs. 67, 68). The phosphorylation from pNPP and Pi requires a reaction of the system with g-strophanthin and magnesium.

The phosphate from AcP,[62,63] pNPP,[64] and Pi[51,66] seems to be bound to the same group on the enzyme as the phosphate from ATP. (It has not been investigated for ITP). The formation of a high energy bond from a relatively low energy substrate as pNPP and from Pi shows that energy for the formation of the bond under these conditions must come from the reaction of the system with g-strophanthin and not from the substrate. The very slow rate of reaction with g-strophanthin suggests that it involves a change in conformation of the system. This may lead to a transformation of "conformational energy" into bond energy and by this to the formation of the high energy phosphate bond.[66]

The formation of the phospho-enzyme with a high energy phosphate bond is thus not specific for a reaction of the system with ATP. There is, however, a specific requirement for ATP for the transport process.[69] AcP which has a high energy phosphate bond, which gives a sodium-dependent phosphorylation of the system, and which is hydrolyzed by the system at a rate which is comparable to the rate of hydrolysis of ATP[70] cannot give a transport of sodium.[71] This shows either that formation of the phospho-enzyme is not enough for the transport process or that ATP can phosphorylate under conditions where AcP cannot. In either case it shows that there must be an effect of ATP on the system which precedes the phosphorylation (see also ref. 72).

There is a high specificity for the binding of ATP to the system, the affinity is at least 2–3 orders of magnitude higher than the affinity for CTP, GTP, and ITP.[27,73] According to the scheme (1)–(4), potassium and ATP exclude each other, which means that TS with potassium, and TS with ATP, must differ in some way (TS_1^* for the ATP form in the following). The discussed difference in the effect of sodium on the reaction of the system with magnesium with and without ATP seems to support this. The hydrolysis of AcP and pNPP is activated by potassium without sodium, which shows that these substrates must bind to the system in the presence of potassium in contrast to ATP. This suggests that AcP does not change the system in the same manner as ATP apparently does.

The change from TS_1 to TS_1^* due to ATP may be the specific effect of ATP and a necessary prerequisite for the transformation of the system from TS_1 to TS_2 when the bond between the γ and β phosphate of ATP is cleaved. The transformation may follow from the cleavage of this bond and not from the phosphorylation as such, and it may be the transformation which specifically requires an effect of ATP, sodium, and magnesium. The phosphorylation may be of importance for stabilizing the system in the TS_2 state until it can react with potassium, and by this be dephosphorylated and return to the TS_1 state.

As it seems to be the step which leads to the formation of the potassium sensitive phospho-enzyme which involves a change in configuration, it

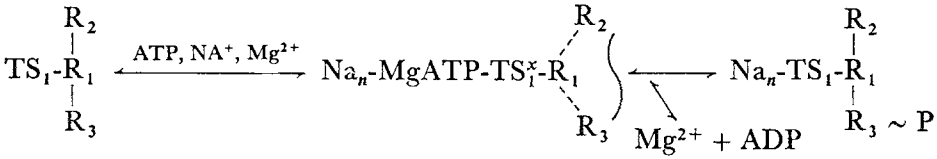
seems likely that this is the step which specifically requires the reaction with ATP. This would exclude the ADP-sensitive phospho-enzyme as an intermediate in the reaction which leads to TS_2, and suggest that it follows from an abortive reaction.

The dephosphorylation by ADP of the "low" magnesium, TS_1, phospho-form suggests that the ΔG for the formation of this phospho-enzyme is close to zero. The lack of reaction of the "high" magnesium form with ADP in spite of a high energy phosphate enzyme bond suggests that formation of the TS_2 phospho-enzyme is an energy-requiring process.

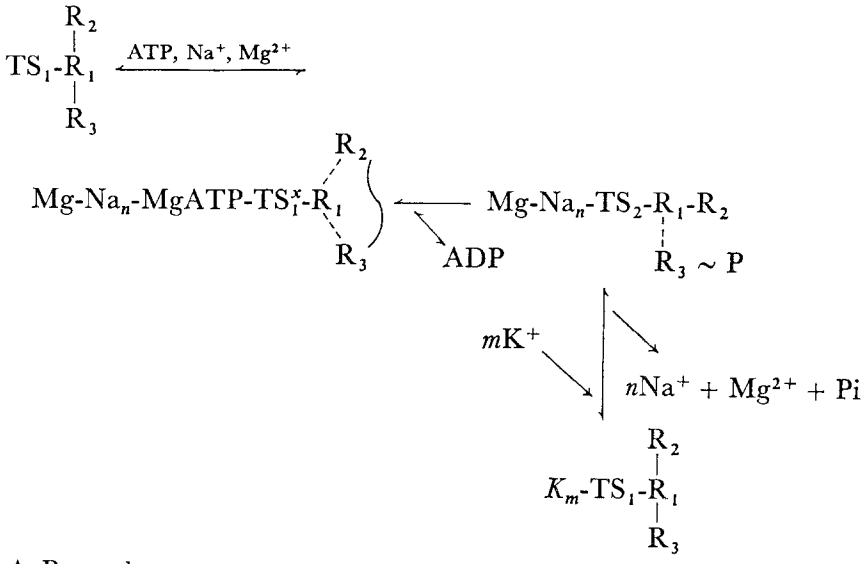
The reaction may be illustrated in a simple way by the following scheme (modified from ref. 74).

R_1, R_2, R_3 illustrate a conformation inside the system. $R_3 \sim R_2$ indicates a change in the distribution of energy inside the system.

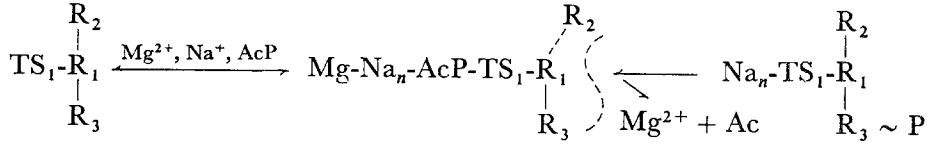
Low Mg^{2+}



High Mg^{2+}

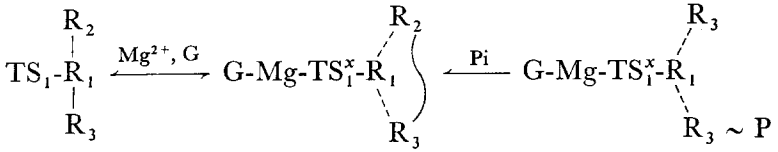


AcP as substrate



The formation of a high energy phosphate bond from low energy sources, Pi, when the system reacts with g-strophanthin may suggest that g-strophanthin mimics an effect of sodium plus ATP.

g-strophanthin



According to the view given above it is the reaction which leads to the phosphorylation and not the phosphorylation as such which is specific for the reaction with ATP and which is important to get the system to act as a transport system.

D. *With Magnesium, ATP, Sodium, and Potassium*

In the previous section the reaction of the system with magnesium, ATP, and sodium has been discussed. It is, however, characteristic for the system that it requires a combined effect of sodium and potassium for the overall hydrolysis of ATP, where sodium activates on the i-sites, and potassium on the o-sites. How is then the relationship between the effect of the two monovalent cations? Is it the reaction of the system with magnesium, ATP, and sodium, which brings it into a state in which it can react with potassium; or is the activation of the catalytic activity with sodium and potassium in the medium due to a combined, simultaneous effect of sodium on the i-sites and potassium on the o-sites? This problem is intimately related to another problem, namely do the sites on the system alternate between the inside and the outside of the membrane, i.e. between an i- and an o-form, a one-unit system; or, do they exist simultaneously, a two-unit system.[75]

There is at present no answer to the problem, and as the interpretation of the effect of sodium plus potassium on the enzyme system differs for a one- and a two-unit system, it may be of interest shortly to discuss both possibilities.

1. *One-unit system.* In a one-unit system the i-sites must be transformed into o-sites and back again as the reaction proceeds. As sodium activates on the i-sites and potassium on the o-sites, this means that the reaction of the system with potassium must follow that of the reaction with sodium. The reaction with sodium leads to the formation of the potassium-sensitive phospho-enzymes. Potassium added to a prephosphorylated enzyme increases the rate of dephosphorylation, which means that the reaction consists of a sodium-dependent phosphorylation followed by a potassium-dependent dephosphorylation.

The concentration of potassium necessary to dephosphorylate the potassium-sensitive enzyme is very low relative to the concentration of sodium in the medium, and it increases with the sodium concentration.[49]

This suggests that the potassium-sensitive phospho-enzyme, Mg-Na_n-$TS_2 \sim P$, is a form of the system in which the sites have a much higher apparent affinity for potassium than for sodium, i.e. the form in which the sites face the outside of the membrane, o-sites.

In the intact cell the transport system can accomplish an exchange of sodium from inside with sodium from outside, a Na:Na exchange, and this is sensitive to oligomycin.[76] Oligomycin increases the sodium-dependent labelling from ATP^{32}, and this seems to be due to a decreased rate of dephosphorylation.[49,62,77–79] As oligomycin has no effect or enhances the sodium-dependent ATP-ADP exchange found when the magnesium concentration is low relative to the ATP concentration,[50,58] it seems to be the dephosphorylation of the potassium-sensitive phospho-enzyme, Mg-Na_n-$TS_2 \sim P$, which is inhibited by oligomycin, and not the dephosphorylation of Na_n-$TS_1 \sim P$. This suggests that it is the inhibition of the dephosphorylation of Mg-Na_n-$TS_2 \sim P$, which leads to an inhibition of the Na:Na exchange, and that this form takes part in the Na:Na exchange. It suggests that the system prior to the formation of the potassium-sensitive phospho-enzyme is in contact with the inside solution.

It was suggested above that the formation of the ADP-sensitive phospho-enzyme was an abortive reaction found when the magnesium concentration was too low, and that this intermediate was not part of the reaction when the magnesium concentration was high enough to give the conformational change of the system from the TS_1 to the TS_2 state, to the potassium-sensitive form. If this is correct, it must be Mg-Na_n-TS_1^*-MgATP which is the form prior to Mg-Na_n-$TS_2 \sim P$, and which is in contact with the inside solution. It leads to the scheme shown in Fig. 4a for the connection between the reaction of the system with ATP and the transport process. In the scheme it is the cleavage of the γ–β phosphate bond of ATP by the system in the form into which it has been brought due to the reaction with ATP, sodium, and magnesium that leads to the conformational change, $TS_1 \rightarrow TS_2$. This gives the transformation of i-sites into o-sites, and by this a translocation of sodium from inside to outside followed by an exchange of sodium for potassium on the o-sites. In Fig. 4b is shown the alternative possibility that the ADP-sensitive phospho-enzyme, $TS_1 \sim P$, is an intermediate in the reaction.

The shift in affinity may mean that for the TS_2 state the equilibrium between the sodium and the potassium form, $TS_2 \rightleftharpoons TS_2'$, is towards the potassium form, TS_2'. The exchange of sodium for potassium from outside is dependent on this equilibrium, and on the K/Na ratio in the external solution. On the potassium form the system is dephosphorylated, and this leads to the back-transformation of the system into the TS_1 state, $TS_2' \rightarrow TS_1'$, with a transport of potassium from outside to inside.

In the scheme shown in Fig. 4, the chemical change is intimately related to the translocation, and to the change from a sodium to a potassium affinity.[80] For a detailed discussion of the scheme, see ref. 75.

It must be emphasized that a translocation from TS_1 to TS_2 in a one-unit system not necessarily means a macroscopic (relative to the dimensions of the membrane) movement of a carrier molecule from the inside to the outside of the membrane and that phosphate is moved from inside to outside. The system may both in the TS_1 and TS_2 state be in

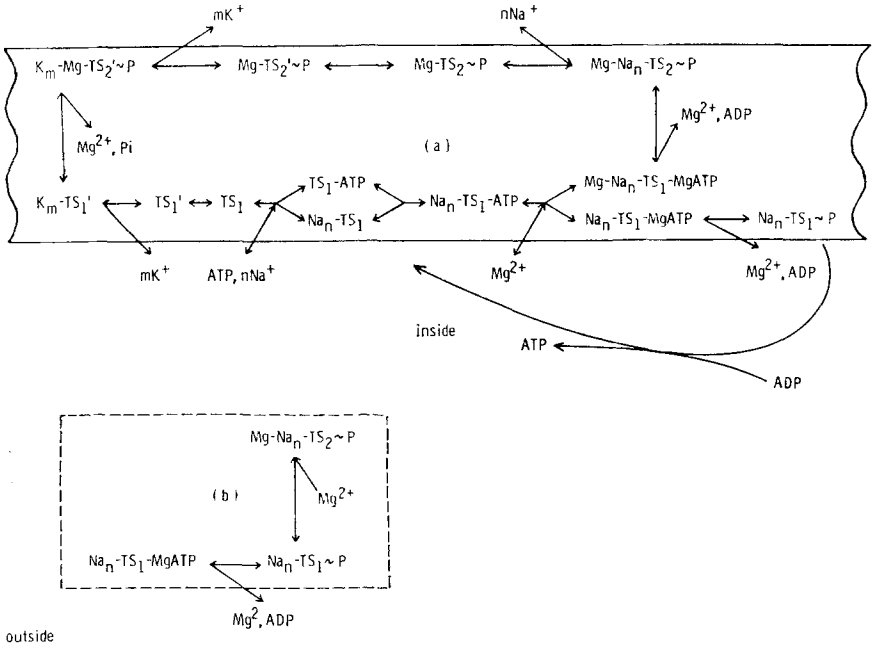


Figure 4. A one-unit model for the transport process. For explanation, see text. Modified from ref. 75.

contact with the inside of the membrane, but in the TS_1 state be able to exchange cations with the internal solution, and in the TS_2 state with the external solution, as for example in an alternating gate system.[81]

2. *Two-unit system.* In a two-unit system, see Fig. 5 there exists at the same time sites for sodium and potassium on the inside and on the outside of the membrane. The double competition between sodium and potassium suggests that each set of sites can exist on a sodium or on a potassium form: $o_S \leftrightharpoons o_P$, and $i_S \leftrightharpoons i_P$, respectively (o for outside, i for inside, S for sodium, and P for potassium). Each of the units accepts more than one cation (*m* and *n* in the figure); assuming that

each of the units accepts only sodium or potassium, i.e. that none of the units exists in a hybrid form (see, however, ref. 82), there are four combinations of the transport system, o_S/i_S, o_S/i_P, o_P/i_S, o_P/i_P, which must exist at the same time in the membrane. The ratio between these forms must depend on a number of factors. One is the built-in differences in affinities for each of the units for sodium and potassium.

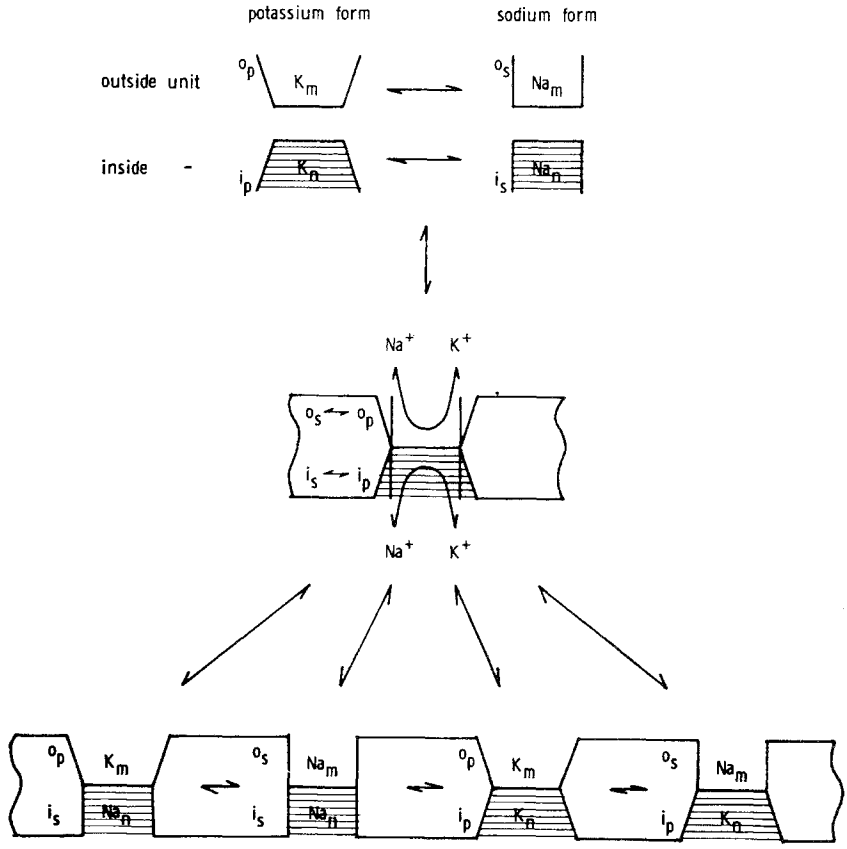


Figure 5. A two-unit model. For explanation, see text.

Another is the ratio between the concentrations of sodium and potassium in the solutions in contact with the sites. A third is the concentration of ATP; according to what has been discussed above, the i-sites on the potassium form, i_P, have a low affinity for ATP or do not react with ATP, while the sodium form, i_S, has a high affinity, which means that ATP will tend to shift the equilibrium towards the formation of is on account of i_P (see Fig. 6). The effect of the K:Na ratio on the equilibrium between the potassium and the sodium form of the o-unit seems to be independent of their effect on the equilibrium between the potassium and sodium form of the i-unit.[83]

The i-unit on the sodium-ATP form complexes magnesium with a very high affinity and reacts with Mg^{2+} with a lower affinity as has been described for a one-unit system. The difference between the reaction with MgATP and MgATP plus Mg^{2+} seems to be a change in conformation which is in some way related to the translocation of the cations (see section V, C). This may for a two-unit system mean that the reaction with Mg^{2+} besides MgATP leads to a change in the interaction between the two units from a state in which the cations on the o-unit do not

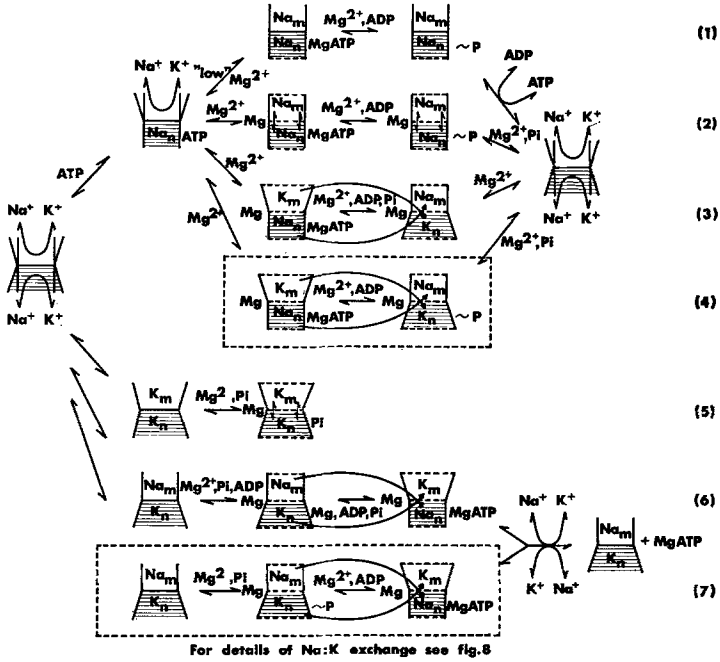


Figure 6. A two-unit model for the transport process. For explanation, see text. Modified from ref. 75.

influence the catalytic activity of the system to a state in which they do, and in which an exchange of the cations in between the two units is made possible. The change in interaction between the two units is shown on Figs. 6, 7, and 8 as a change from a situation where the two units are separated by a full-drawn line to a situation where they are separated by a dashed line, named a non-interacted and an interacted state, respectively, in the following; this indicates nothing about the molecular events—it is used to describe states which react differently. The dashed line on the surface of the units indicates a decreased exchange of the cations between the units and the surroundings.

For a one-unit system, the potassium-sensitive phospho-enzyme, $TS_2 \sim P$, formed with sodium but no potassium in the medium must be part of the reaction with sodium plus potassium, while this may or may not be the case for the ADP-sensitive phospho-enzyme, $TS_1 \sim P$.

If it is a two-unit system, the formation of $TS_1 \sim P$ and $TS_2 \sim P$ with sodium without potassium, i.e. with the system on the Na^o_m/Na^i_n form could be parallel reactions, (1) and (2) in Fig. 6, or consecutive reactions, Fig. 7, as for the one-unit system. With sodium plus potassium the hydrolysis of ATP could be due to a consecutive reaction in which the i-sites react with sodium, and this is followed by a reaction of the o-sites with potassium, Fig. 7; or, to a combined, simultaneous effect of potassium on the o-sites and of sodium on the i-sites, Fig. 6.

In the consecutive reaction shown in Fig. 7, the formation of $TS_1 \sim P$ is due to an effect of sodium on the i-sites and when $TS_1 \sim P$ reacts with Mg^{2+}, it is transformed into $TS_2 \sim P$, i.e. into the state of interaction between the two units in which the cations on the o-sites influence the rate of hydrolysis of the phospho-enzyme.

Another possibility (not shown) could be that the formation of $TS_2 \sim P$ requires a combined effect of sodium on the i- and the o-sites, and that the formation of $TS_2 \sim P$ leads to a shift of the o-unit from a sodium to a potassium form with a following exchange of sodium for

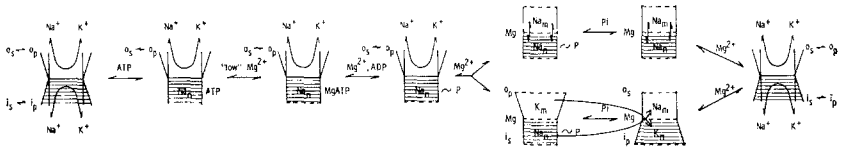


Figure 7. A two-unit model for the Na:K exchange with the ADP- and potassium-sensitive phospho-enzyme as an intermediate. For explanation, see text; see also Fig. 6.

potassium on the o-site, $Na^o_m/Na^i_n \rightarrow K^o_m/Na^i_n$, followed by a dephosphorylation and exchange of the cations in between the two units as shown in Fig. 7.

The consecutive reaction seems, however, to be ruled out by the observation that potassium in activating concentrations decreases the apparent affinity for magnesium plus ATP. In experiments where magnesium and ATP were kept at a constant 1 : 1 ratio, it was found that the concentration necessary for half saturation of the $(Na^+ + K^+)$-dependent hydrolysis by the enzyme system increased with the potassium concentration[84] (see also ref. 85). The effect of potassium was seen in the range in which potassium activates (the right part of the curve in Fig. 1) which suggests that it is an effect of potassium on the o-sites. It can therefore not be explained by the discussed effect of potassium on the i-sites on the affinity for ATP. A decrease in the affinity for magnesium plus ATP on the inside, on the i-unit, due to an effect of potassium on the o-sites may suggest that potassium reacts with the o-sites before the γ–β bond of ATP is cleaved. This seems to rule out the consecutive reaction described above (cf. Fig. 7). It suggests that the reaction of the system on the K^o_m/Na^i_n form follows a pathway which is different from that of the system on the Na^o_m/Na^i_n form, Fig. 6, and that

the hydrolysis of ATP is due to a combined, simultaneous effect of potassium on the o-sites and sodium on the i-sites. The lower affinity for magnesium plus ATP may be due to a way of interaction between the units on the o_P/i_S form which is different from that on the i_S/i_P form.

A different pathway for the reaction on the Na^o_m/Na^i_n and the K^o_m/Na^i_n form raises the question whether the intermediate formed with the system on the Na^o_m/Na^i_n form, $TS_2 \sim P$, is also part of the reaction with the system on the K^o_m/Na^i_n form? The answer depends on the answer to another question: is the formation of $TS_2 \sim P$ with the system on the Na^o_m/Na^i_n form due to a combined effect of sodium on the o- and i-sites, or is it enough that there is sodium on the i-sites? If it requires a combined effect of sodium on the two units, $TS_2 \sim P$ cannot be part of the reaction with the system on the K^o_m/Na^i_n form. A decreased labelling found with sodium plus potassium must then be due to a decrease in the amount of the system on the Na^o_m/Na^i_n form.

If sodium on the i-sites is enough for the formation of $TS_2 \sim P$, what happens then when the system is on the K^o_m/Na^i_n form? The answer depends on the answer to another question: when the system on the Na^o_m/Na^i_n form is prephosphorylated and potassium is added, is the increased rate of dephosphorylation then due to an exchange of sodium for potassium on the o-site or on the i-site? Potassium has the effect in such low concentrations relative to the concentration of sodium that it suggests that it is an effect of potassium on the o-sites.[49] If this is the case, it is difficult to see what the result would be of an effect of sodium on the i-sites which gives a high rate of hydrolysis of the γ–β bond of ATP, and which tends to form a bond between the γ-phosphate and the enzyme and of potassium on the o-sites which tends to increase the rate of hydrolysis of the bond between the γ-phosphate and the enzyme. Will it be a phosphorylation, or will it be a hydrolysis of ATP without formation of a covalent bond between the enzyme and the phosphate ((3) in Fig. 6)?

If, on the other hand, the effect of potassium on the dephosphorylation of a prephosphorylated enzyme is due to an exchange of sodium for potassium on the i-sites, the phosphorylation found with the Na^o_m/Na^i_n form of the system could be part of the reaction with the system on the K^o_m/Na^i_n form, even if the two reactions follow a different pathway ((2) and (4) in Fig. 6).

A different pathway for the hydrolysis of ATP on the Na^o_m/Na^i_n and on the K^o_m/Na^i_n form with different affinities of the two forms for Mg^{2+} and ATP may explain why the same system seems to behave as two different systems, one found with sodium and no potassium, the other found with sodium and potassium.[86] (For a detailed discussion of the two-unit model, see ref. 75.)

It is not possible from our present knowledge to decide between the different possibilities, a consecutive reaction, Fig. 7, a simultaneous

reaction without formation of a phospho-enzyme, (3) in Fig. 6, a simultaneous reaction with formation of a phospho-enzyme, (4) in Fig. 6. That means that it is neither possible to tell what it is that gives the translocation of the cations. Whether it is the hydrolysis of ATP without the phosphorylation, (3) in Fig. 6, the formation of a phosphorylated intermediate, (4) in Fig. 6, or the dephosphorylation of the prephosphorylated system, Fig. 7. Nor is it possible to tell what it is that gives the shift in the affinity of the o-sites which is necessary for the exchange of the cations.

The number of sodium ions transported per ATP hydrolyzed is about 3, while the number of potassium ions transported is lower.[4] This may mean that n is 3 and m a lower number (Figs. 5–8). Another possibility is that m and n are not identical with the number of cations transported. When ATP is hydrolyzed, and the o-unit is changed from o_P to o_S, and

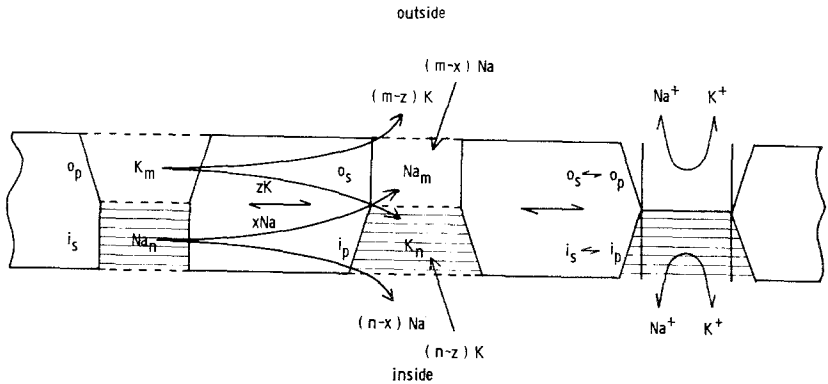


Figure 8. A model to describe the variation in the Na:K coupling ratio for a two-unit model. For explanation, see text. Modified from ref. 75.

the i-unit from i_S to i_P (see Fig. 8),[75] there may be an exchange of cations both in between the two units and between the units and the external and internal solution. Let for example m and n in Fig. 8 be equal to 4, x to 3, and z to 2. When the affinity of the two units are changed, there may be a flow of 3 sodium ions (x) from i to o, of 1 sodium ion (n–x) from i to the internal solution, and of 1 sodium ion (m–x) from the external solution to the o-unit. Simultaneously, there may be a flow of two potassium ions (z) from the o- to the i-unit, of two potassium ions (m–z) from the o-unit to the external solution, and of two potassium ions (n–z) from the internal solution to the i-unit. By this the four sites on the o-unit are filled up with sodium, and on the i-unit with potassium in spite of a transfer of only 3 sodium ions from i to o, and 2 potassium ions from o to i. When sodium on the o-unit in the following step in the non-interacted state is exchanged for

potassium from the outside solution and potassium on the i-unit with sodium from the inside solution, the net result has been a transfer of 3 sodium out and 2 potassium in. This will besides a transport of the cations give an effect on the potential across the membrane, an electrogenic pump. *m* needs not be identical with *n*, and they can take any number equal to or higher than 3. The net number of cations exchanged between the two units depends on the ratio between the resistances for the flow of the cations in between the units and between the units and the external and internal solutions, respectively, and on the electrochemical gradient between the units and the external and internal solutions. The coupling ratio can vary without a variation of the number of sites for the cations on the units, *m* and *n*. For *z* equal to 0, the system gives a sodium efflux which is not coupled to a potassium influx, but is activated by potassium from outside.

VI. *Fluxes of Sodium and Potassium in Intact Cells in Relation to the Transport Models*

Besides the sodium efflux which is chemically coupled to a potassium influx there are a number of other carrier-mediated fluxes of sodium and potassium across the cell membrane. One is a sodium efflux which requires potassium in the external medium, but is not chemically coupled to a potassium influx, an electrogenic pump;[87,88] another is a sodium efflux which is coupled to a sodium influx, a Na:Na exchange.[12,71,89–95] A third is a sodium efflux seen without sodium and potassium in the external medium.[90,96] A fourth is a potassium influx which can either be coupled to a sodium influx, a reversal of the pump,[7,76,97] or to a potassium influx, a K:K exchange.[7]

The ratio between these fluxes depends in a complicated and only partly understood manner on the ratio between potassium and sodium in the external solution,[92,93] and in the internal solution,[71,92,94] and on the internal concentrations of ATP, ADP, and Pi,[92,98–100] and maybe also Mg^{2+}. It is common for all these fluxes that they are inhibited by cardiac glycosides. It seems therefore likely that they are all due to the same transport system in the cell membrane, and as the $(Na^+ + K^+)$-activated enzyme system is specifically inhibited by cardiac glycosides, it seems likely that this is the common transport system for the different cardiac glycoside-sensitive fluxes of sodium and potassium.

This means that the system must have a high degree of flexibility. The Na:K coupling can be switched over to a Na:Na or to a K:K coupling, and the Na:K coupling ratio may vary. How does this fit with the two discussed transport models which are mainly based on observations on the $(Na^+ + K^+)$-activated enzyme system in the test tube?

A. *Sodium-potassium Coupling*

The sodium pump can generate a potential across the cell membrane.[87,88] This effect requires potassium (or another of the cations which can activate the sodium pump) in the external solution, and it is inhibited by g-strophanthin. With a high internal sodium concentration, a hyperpolarization can be seen with a membrane potential more negative than the potassium equilibrium potential, and which is apparently not due to a depletion of potassium from the external surface by a neutral pump. It suggests that there is a transport of sodium outwards which is not chemically coupled to an inward transport of potassium, or to an outward transport of an anion. It means that the outward transport of sodium gives a transfer of net electrical charge across the membrane, and that the sodium pump can act as an electrogenic pump.

This fits with the observation on red blood cells that the number of sodium ions transported out per ATP hydrolyzed is higher than the number of potassium ions transported in, namely about 3:2.[4]

A problem is whether the 3:2 ratio is a fixed ratio found under all conditions and in all cells, and furthermore, whether this means that the pump in each cycle transports three sodium out and two potassium in, or that the pumping consists of a mixture of a neutral 3:3 pump and an electrical pump in which three sodium ions are transported out without an inward transport of potassium, but activated by potassium from outside.

Or, is the coupling ratio variable, and if so, is it a variation in the ratio between a neutral and an electrogenic pumping; or is the sodium pump more flexible and can pump sodium and potassium with a variable chemical coupling ratio, which is a function of factors like membrane potential and electrochemical potential gradients for the ions between the membrane phase and the solutions?

There is no definite answer to this problem. In experiments on the electrogenic effect of the sodium pumping in stretch receptors[101] and ganglion cells[102] under conditions where the internal sodium concentration was high, it was found that about $\frac{1}{4}$–$\frac{1}{3}$ of the sodium was extruded uncoupled in agreement with the Na:K coupling ratio of 3:2 found in red cells. In giant axons, with a high internal sodium concentration, the Na:K coupling ratio was found to be of the same order, namely 3:1[71] and 2:1.[94] If, however, the internal sodium concentration was decreased, the coupling ratio increased towards 1:1 at a low internal sodium concentration, suggesting that the coupling ratio may vary as a function of the internal sodium concentration.

A fixed coupling ratio can be explained from both the one- and the two-unit model. A variation in the coupling ratio is simpler to explain from the two-unit model (Fig. 8), and especially an efflux of sodium which is activated by potassium from outside, but which is not

chemically coupled to an influx of potassium (if it exists). In the two-unit model the coupling ratio depends on the resistances for the flow of the cations inside the units and between the units and the external and internal solutions, respectively; and on the electrochemical potential gradients for the ions between the units and the solutions.

B. *Na : Na Exchange*

According to Garrahan and Glynn,[76] at least four features of the g-strophanthin-sensitive Na:Na exchange have to be taken into consideration when discussing a hypothetical model for the transport.

1. ATP is necessary for the exchange to take place in spite of low or no hydrolysis of ATP.
2. The exchange increases in rate with increasing external sodium concentration over a wide range and under conditions where the external sodium concentration is higher than the internal sodium concentration.
3. With a high internal ATP concentration relative to the internal Pi, there will be no exchange when the internal sodium is high, only with a low internal sodium and a high internal potassium concentration. With a high internal sodium, an increase in the internal Pi relative to the ATP will increase the Na:Na exchange.
4. The Na:Na exchange is oligomycin-sensitive.

And according to Glynn and Hoffman:[100]

5. ADP is necessary.

1. *One-unit system.* The requirement for ADP for the Na:Na exchange and the low hydrolysis of ATP due to this exchange seems to exclude that the exchange reaction can be due to a forward reaction of the system in which the dephosphorylation of the system in the TS_2 state is due to an effect of sodium from outside, instead of potassium, followed by a back-translocation on the sodium form.

It suggests the reaction

$$Mg\text{-}Na_n\text{-}TS_1\text{-}MgATP \leftrightharpoons Mg\text{-}Na_n\text{-}TS_2 \sim P + ADP + Mg^{2+}$$

as responsible for the exchange reaction (Fig. 4a).

Or, in the reaction scheme where the ADP-sensitive phosphoenzyme is an intermediate in the reaction (Fig. 4b), the reaction[100]

$$Na_n\text{-}TS_1 \sim P + Mg^{2+} \leftrightharpoons Mg\text{-}Na_n\text{-}TS_2 \sim P.$$

A Na:Na exchange due to these reactions requires ATP, but there is no net hydrolysis of ATP (requirement 1). They lead to a shift in affinity which makes it possible to explain requirement 2. They are oligomycin-sensitive (requirement 4). Internal sodium, magnesium, and ATP tend to shift the equilibrium towards the translocated

state, and ADP towards the non-translocated state; it seems therefore possible to explain the effect on the Na:Na exchange of a decrease in internal sodium concentration, a decrease in internal ATP and an increase in internal ADP concentration from their effect on the equilibrium of the reaction (requirements 3 and 5). It is, however, difficult to explain the effect of high internal Pi.

2. *Two-unit system.* With sodium and potassium in the external medium, a decrease in the ATP/ADP × Pi concentration leads to a decrease in the potassium sensitivity of the sodium efflux and apparently to an increase in the Na:Na exchange and a decrease in the potassium influx.[98,99] This observation is simpler to explain from a two-unit model than from the one-unit model shown in Fig. 4 (for discussion of this problem for a one-unit model, see ref. 103).

The observations on the effect of potassium on the requirement for magnesium plus ATP suggested that the Na^o_m/Na^i_n form of the system has a higher affinity for magnesium and ATP than the K^o_m/Na^i_n form. A decrease in the ATP/ADP × Pi ratio at a given potassium/sodium ratio in the external solution therefore tends to shift the equilibrium from the interacted state of the Mg-K^o_m/Na^i_n-MgATP form towards the interacted state of the Mg-Na^o_m/Na^i_n-MgATP form of the system; this may explain the apparent decrease in potassium sensitivity of the sodium efflux, and an increase in Na:Na efflux (see below).

It may also explain why the addition of potassium to an external medium containing sodium decreases the g-strophanthin-sensitive sodium efflux, when the ATP/ADP × Pi ratio is low.[92] Without potassium in the external solution, a certain part of the system is on the Na^o_m/Na^i_n form, and this may as discussed below give a Na:Na exchange, when the ATP/ADP × Pi ratio is low. When potassium is added to the external solution, a part of the Na^o_m/Na^i_n form is transferred into the K^o_m/Na^i_n form. This gives a decrease in the sodium efflux coupled to a sodium influx. But due to the low ATP/ADP × Pi ratio, the equilibrium of the K^o_m/Na^i_n form is towards the non-interacted state, which means that the decrease in sodium efflux coupled to a sodium influx will not, or only to a lower extent, be replaced by a sodium efflux coupled to a potassium influx.

Removal of potassium from the external solution shifts the equilibrium towards Na^o_m/Na^i_n on account of K^o_m/Na^i_n. In the interacted state of the Na^o_m/Na^i_n form, the system may exchange sodium in between the two units (see (2) in Fig. 6), and the forward and backward reaction of Mg-Na^o_m/Na^i_n-MgATP $\leftrightharpoons$ Ma-$Na^o_m/Na^i_n \sim$ P + ADP + Mg^{2+} may give a Na:Na exchange as the parallel reaction in the one-unit system.

In the two-unit system there are, however, at least two other ways to explain the Na:Na exchange and the requirement for ADP.

Assuming that sodium in the interacted state can be exchanged not only in between the two units, but also between the units and the

surroundings, the forward reaction with a low rate of turnover, because of a high concentration of ADP, may give a high rate of exchange.

A more complicated reaction would be that the catalytic reaction of $Mg\text{-}Na^{o}_{m}/Na^{i}_{n}\text{-}MgATP$ ((2) in Fig. 6) in the interacted state tends to transform the i-unit from i_S to i_P, as is the case when the system reacts with ATP on the K^{o}_{m}/Na^{i}_{n} form. As there is no potassium on the o-unit to be exchanged for sodium on the i-unit, sodium will hinder this, and the result of the reaction is therefore not a hydrolysis of ATP, but the phosphorylation with formation of the potassium-sensitive phospho-enzyme. This phospho-enzyme may react with ADP and form ATP, but only if i_S is transformed into i_P, i.e. if sodium on the i-unit is exchanged for potassium from inside. This reaction is supported by a high internal ADP and a high internal potassium/sodium ratio. The dephosphorylation leads to a transformation into the non-interacted state. It will give a Na:Na exchange, which is increased by a high internal ADP and a high internal K/Na ratio, and which requires ATP with no net hydrolysis of ATP, and is oligomycin-sensitive. It would mean that the potassium-sensitive phospho-enzyme cannot be an intermediate in the reaction on the K^{o}_{m}/Na^{i}_{n} form. Furthermore, that the dephosphorylation of the potassium-sensitive phospho-enzyme besides potassium requires ADP, and is due to an effect of potassium on the i-unit and not on the o-unit!

There is a g-strophanthin-insensitive sodium efflux which, according to Hoffman and Kregenow,[104] is on another transport system (pump II). Brinley and Mullins[95] showed that strophanthidin increases the sodium efflux when the ATP concentration is very low, but it decreases the influx when the ATP concentration is high. The increase and decrease occur to the same level of efflux. As pointed out by the authors, this suggests that the cardiac glycoside-sensitive transport system is responsible for the cardiac glycoside-insensitive sodium efflux. It suggests that strophanthidin blocks the system in a state in which it can accomplish a low sodium efflux (Na:Na exchange?). The increase in sodium efflux by strophanthidin when the concentration of ATP is low and the decrease when the concentration of ATP is high, suggest that this state is an intermediate in the turnover of the system. It suggests that the reaction with strophanthidin with a low concentration of ATP leads to a shift in equilibrium from a state in which the system cannot give a sodium efflux, and which does not react with strophanthidin, the non-interacted state, towards a state in which it can give a sodium efflux, and which can react with strophanthidin, the interacted state. With the higher concentration of ATP, g-strophanthin blocks the system in the same state as with the low concentration of ATP. By this it blocks the turnover and decreases the sodium efflux to the same level as found with strophanthidin and the low concentration of ATP.

C. *Reversal of the Sodium Pump*

Under certain conditions, namely with external sodium, but no external potassium, and with a high internal potassium concentration and a low ATP/ADP × Pi ratio, it is possible to drive the cation pump backwards and form ATP from ADP and Pi.[7,76,97]

In the one-unit model it can be explained by reversal of the forward reaction.

In the two-unit model, the equilibrium of the o-unit is towards the potassium form, o_P, but with sodium and no potassium, the unit will be on the sodium form, o_S. When this form interacts with the i-unit on the potassium form, i_P, in the presence of magnesium, ATP, and Pi, there may be a tendency for the cations to be exchanged in between the two units with a shift in the equilibrium towards o_P/i_S, and with a formation of ATP ((6) and (7), cp. (3) and (4) respectively, in Fig. 6).

D. *K:K Exchange*

Under certain conditions, namely with a high internal concentration of potassium and Pi, a low concentration of ATP, and with potassium in the external medium, there seems to be a g-strophanthin-sensitive potassium efflux which is coupled to a potassium influx, a K:K exchange.[7]

In the one-unit system, the K:K exchange can be explained by a reversal of the last step of the transport process. This step is related to the potassium entry mechanism, which may explain why Pi is necessary.

In the two-unit system, the K:K exchange can be explained by a shuttling of the system on the K^o_m/K^i_n form between the non-interacted and interacted states ((5) in Fig. 6); this does not represent a reversal of the system in the same sense as in the one-unit system, but a reaction of the system on the K^o_m/K^i_n form independent of the reaction for the potassium-coupled sodium efflux.

VII. *Conclusion*

In the preceding section the sequence of the steps in the reaction of the $(Na^+ + K^+)$-activated enzyme system has been discussed, and an attempt has been made to relate them to the transport of cations across the cell membrane and from this to formulate a model for the transport process.

The result of this has been two principally different models, for both of which it must be emphasized that they are based on a number of assumptions, and must only be taken as suggestions. It is not possible from our present knowledge to decide whether the transport process can be described by a one-unit or by a two-unit model. The two-unit model seems to have a few advantages over the one-unit model. It is

simpler to explain a variable ratio between the number of sodium and potassium ions transported, and also an external potassium activation of a sodium efflux without an influx of potassium. Furthermore, that potassium from outside influences the affinity for magnesium and ATP on the inside, and also that a decrease in the ATP/ADP × Pi ratio decreases the sensitivity of the sodium efflux towards potassium from the external solution. Each of these problems can, however, also be explained from a one-unit model.

There is, however, one set of experimental results which lends support to a two-unit system, and which only under special conditions can be explained from a one-unit system. That is the observations by Hoffman and Tosteson on sheep red cells that the apparent affinity of external potassium and of internal sodium for the g-strophanthin-sensitive sodium efflux and potassium influx is independent of the internal and external Na/K ratios, respectively.[83]

Experiments on the effect of ATP on the phosphatase activity of the system raise another problem which has not been discussed. The $(Na^+ + K^+)$-activated enzyme system can apparently hydrolyze pNPP in the presence of magnesium and potassium.[53, 62–64, 105–113] The effect of potassium on the hydrolysis of pNPP is, however, influenced by ATP and sodium.[108–112] Does that mean that the system binds ATP at a modifier site, and pNPP at a catalytic site?[111] And does that mean that the system with ATP as substrate binds two ATP molecules at two different sites, one which has a modifying effect on the hydrolysis, and another which is hydrolyzed? (See also ref. 27.)

Or, is the effect of ATP on the hydrolysis of pNPP due to a consecutive reaction of the system with ATP and pNPP, i.e. the reaction with ATP leads to a phosphorylation, and this is followed by a reaction with pNPP on the ATP-site? Or, can pNPP induce a change in the catalytic site which allows binding of both ATP and pNPP to the same site, while without pNPP only one ATP molecule is bound?

At present there is no definite answer to the problem about a one- or a two-unit system, nor to the problem about one or two sites for ATP on the system. This tells that our knowledge about the sequence of the steps in the reaction is not detailed enough. And, apart from that, we have nearly no information about the molecular structure and the molecular events related to the steps in the reaction. Where in the membrane is the system located, on the inside or on the outside or all the way through the membrane? How far are the cations transported on the system? How do the units discriminate between the cations? Do they behave as ion exchangers, or are there specialized structures of the type which has been used in bilayer studies to discriminate between sodium and potassium? This and many other questions have to be answered before we are able to understand the transport process.

Reference

1. J. C. Skou, *Physiol. Rev.*, **45** (1965) 596.
2. E. Heinz, *A. Rev. Physiol.*, **29** (1967) 21.
3. R. W. Albers, *Ann. Rev. Biochem.*, **36** (1967) 727.
4. I. M. Glynn, *Br. Med. Bull.*, **24** (1968) 165.
5. R. Whittam and K. P. Wheeler, *Ann. Rev. Physiol.*, **32** (1970) 21.
6. S. L. Bonting, in: *Membranes and Ion Transport*, Vol. 1, E. E. Bittar (ed.), Wiley-Interscience, New York, 1970, p. 257.
7. I. M. Glynn and V. L. Lew, *J. Gen. Physiol.*, **54** (1969) 289s.
8. J. C. Skou, *Biochim. Biophys. Acta*, **23** (1957) 394.
9. I. M. Glynn, *J. Physiol.* (Lond.), **160** (1962) 18P.
10. P. C. Laris and P. E. Letchworth, *J. Cell. Comp. Physiol.*, **60** (1962) 229.
11. R. Whittam, *Biochem. J.*, **84** (1962) 110.
12. P. J. Garrahan and I. M. Glynn, *J. Physiol. (Lond.)*, **192** (1967) 217.
13. P. Leth Jørgensen and J. C. Skou, *Biochim. Biophys. Acta*, **233** (1971) 366.
14. O. J. Møller, *Exp. Cell Res.*, **68** (1971) 347.
15. J. Rostgaard and O. J. Møller, *Exp. Cell Res.*, **68** (1971) 356.
16. J. C. Skou, *Biochim. Biophys. Acta*, **58** (1962), 314.
17. T. Nakao, Y. Tashima, K. Nagano and M. Nakao, *Biochem. Biophys. Res. Comm.*, **19** (1965) 755.
18. J. C. Skou, *Biochem. Biophys. Res. Comm.*, **10** (1963) 1.
19. H. Matsui and A. Schwartz, *Biochim. Biophys. Acta*, **128** (1966) 380.
20. P. Leth Jørgensen, J. C. Skou and L. P. Solomonson, *Biochim. Biophys. Acta*, **233** (1971) 381.
21. S. Uesugi, N. C. Dulak, J. F. Dixon, T. D. Hexum, J. L. Dahl, J. F. Perdue and L. E. Hokin, *J. Biol. Chem.*, **246** (1971) 531.
22. J. Kyte, *J. Biol. Chem.*, **246** (1971) 4157.
23. D. W. Towle and J. H. Copenhaver, Jr., *Biochim. Biophys. Acta*, **203** (1970) 124.
24. H. Bader, R. L. Post and G. H. Bond, *Biochim. Biophys. Acta*, **150** (1968) 41.
25. R. W. Albers, G. J. Koval and G. J. Siegal, *Mol. Pharmacol.*, **4** (1968) 324.
26. J. G. Nørby and J. Jensen, *Biochim. Biophys. Acta*, **233** (1971) 104.
27. C. Hegyvary and R. L. Post, *J. Biol. Chem.*, **246** (1971) 5234.
28. O. Hansen, J. Jensen and J. G. Nørby, *Nature, New Biol.*, **234** (1971) 122.
29. J. C. Ellory and R. D. Keynes, *Nature*, **221** (1969) 776.
30. O. Hansen, *Biochim. Biophys. Acta*, **233** (1971) 122.
31. P. F. Baker and J. S. Willis, *Nature*, **226** (1970) 521.
32. M. Nakao, K. Nagano, T. Nakao, N. Mizuno, Y. Tashima, M. Fujita, H. Maeda and H. Matsudaira, *Biochem. Biophys. Res. Comm.*, **29** (1967) 588.
33. G. R. Kepner and K. J. Maccy, *Biochem. Biophys. Res. Comm.*, **30** (1968), 582.
34. J. Kyte, *Biochem. Biophys. Res. Comm.*, **43** (1971) 1259.
35. B. Roelofsen, R. F. A. Zwaal and L. L. M. van Deenen, in "Membrane-Bound Enzymes", G. Porcellati and F. di Jeso (eds.), *Adv. in Exp. Med. Biol.*, Vol. 14, Plenum Press, New York, 1971, p. 209.
36. P. Emmelot and C. J. Bos, *Biochim. Biophys. Acta*, **150** (1968) 341.
37. H. J. Schatzmann, *Nature*, **196** (1962) 677.
38. T. Ohnishi and H. Kanamura, *J. Biochem.*, **56** (1964) 377.
39. J. C. Skou, in: *Transport and Metabolism*, Vol. 1, A. Kleinzeller and A. Kotyk (eds.), Academic Press, New York, 1961, p. 228.
40. G. Hegyvary and R. L. Post, in: *The Molecular Basis of Membrane Function*, D. C. Tosteson (ed.), Prentice Hall, New Jersey, 1969, p. 519.
41. Y. Israel, in: *The Molecular Basis of Membrane Function*, D. C. Tosteson (ed.), Prentice Hall, New Jersey, 1969, p. 529.
42. K. P. Wheeler and R. Whittam, *J. Physiol. (Lond.)*, **207** (1970) 303.
43. R. Tanaka and L. G. Abood, *Arch. Biochem. Biophys.*, **108** (1964) 47.
44. R. Tanaka and K. P. Strickland, *Arch. Biochem. Biophys.*, **111** (1965) 583.
45. R. Tanaka, *J. Neurochem.*, **16** (1969) 1301.
46. L. J. Fenster an J. H. Copenhaver, Jr., *Biochim. Biophys. Acta*, **137** (1967) 406.
47. R. Tanaka and T. Sakamoto, *Biochim. Biophys. Acta*, **193** (1969) 384.
48. K. A. Karlsson, B. E. Samuelson and G. O. Steen, *J. Membrane Biol.*, **5**, (1971) 169.
49. J. C. Skou and C. Hilberg, *Biochim. Biophys. Acta*, **185** (1969) 198.
50. S. Fahn, G. J. Koval and R. W. Albers, *J. Biol. Chem.*, **241** (1966) 1882.
51. R. L. Post, S. Kume, T. Tobin, B. Orcutt and A. K. Sen, *J. Gen. Physiol.*, **54** (1969) 306s.
52. J. C. Skou, K. Butler and O. Hansen, *Biochim. Biophys. Acta*, **241** (1971) 443.
53. P. J. Garrahan, M. I. Pouchan and F. Rega, *J. Physiol.* (Lond.), **202** (1969) 305.
54. L. E. Hokin, P. S. Sastry, P. R. Galsworthy and A. Yoda, *Proc. Nat. Acad. Sci. U.S.A.*, **54** (1965) 177.
55. K. Nagano, T. Kanazawa, N. Mizuno, Y. Tashina, T. Nakao and M. Nakao, *Biochem. Biophys. Res. Comm.*, **19** (1965) 759.

56. H. Bader, A. K. Sen and R. L. Post, *Biochim. Biophys. Acta*, **118** (1966) 106.
57. S. Fahn, G. J. Koval and R. W. Albers, *J. Biol. Chem.*, **243** (1968) 1993.
58. R. Blostein, *J. Biol. Chem.*, **245** (1970) 270.
59. S. Fahn, M. R. Hurley, G. J. Koval and R. W. Albers, *J. Biol. Chem.*, **241** (1966) 1890.
60. W. Schoner, R. Beusch and R. Kramer, *European J. Biochem.*, **7** (1968) 102.
61. G. H. Bond, H. Bader and R. L. Post, *Fed. Proc.*, **25** (1966) 567.
62. Y. Israel and E. Titus, *Biochim. Biophys. Acta*, **139** (1967) 450.
63. G. H. Bond, H. Bader and R. L. Post, *Biochim. Biophys. Acta*, **241** (1971) 57.
64. C. E. Interrusi and E. Titus, *Mol. Pharmacol.*, **6** (1970) 99.
65. G. E. Lindenmayer, A. H. Langhfer and A. Schwartz, *Arch. Biochem. Biophys.* **127** (1968) 187.
66. G. F. Siegel, G. F. Koval and R. W. Albers, *J. Biol. Chem.*, **244** (1969) 3264.
67. G. Sachs, J. P. Rose and B. I. Hirschowitz, *Arch. Biochem. Biophys.*, **119** (1967) 277.
68. W. F. Dudding and C. G. Winther, *Biochim. Biophys. Acta*, **241** (1971) 650.
69. J. F. Hoffman, *Circulation*, **26** (1962) 1201.
70. H. Bader and A. K. Sen, *Biochim. Biophys. Acta*, **118** (1966) 116.
71. L. F. Mullins and F. J. Brinley, *J. Gen. Physiol.*, **53**, (1969), 704.
72. J. C. Skou, *Biochim. Biophys. Acta*, **42** (1960) 6.
73. J. Jensen and J. G. Nørby, *Biochim. Biophys. Acta*, **233** (1971) 395.
74. J. C. Skou, in: *Biomembranes*, L. A. Manson (ed.), Vol. 2, Plenum Publishing Corporation, New York, 1971, p. 165.
75. J. C. Skou, in: *Current Topics in Bioenergetics*, Vol. 4, D. R. Sanadi (ed.), Academic Press, New York, 1971, p. 357.
76. P. J. Garrahan and I. M. Glynn, *J. Physiol. (Lond.)*, **192** (1967) 237.
77. S. Fahn, G. J. Koval and R. W. Albers, *J. Biol. Chem.*, **243** (1968) 1993.
78. R. Whittam, K. P. Wheeler and A. Blake, *Nature*, **203** (1964) 720.
79. N. Gruener and Y. Avi-Dor, *Biochem. J.*, **100** (1966) 762.
80. P. Mitchell, *Advances in Enzymology*, F. F. Nord (ed.), Vol. 29, Interscience Publishers, New York, 1967, p. 33.
81. O. Jardetzky, *Nature*, **211** (1966) 969.
82. H. W. Middleton, *Arch. Biochem. Biophys.*, **136** (1970) 280.
83. P. G. Hoffman and D. C. Tosteson, *J. Gen. Physiol.*, **58** (1971) 438.
84. J. D. Robinson, *Biochemistry*, **6** (1967) 3250.
85. R. L. Post, A. K. Sen and R. S. Rosenthal, *J. Biol. Chem.*, **240** (1965) 1437.
86. A. H. Neufeld and H. M. Levy, *J. Biol. Chem.* **244** (1969) 6493.
87. R. P. Kernan, in: *Membrane and Ion Transport*, Vol. 1, E. E. Bittar (ed.), Wiley-Interscience, New York, 1970, p. 395.
88. J. M. Ritchie, in: *Current Topics in Bioenergetics*, Vol. 4, R. D. Sanadi (ed.), Academic Press, New York, 1971, p. 327.
89. P. C. Caldwell, A. L. Hodgkin, R. D. Keynes and T. I. Shaw, *J. Physiol. (Lond.)*, **152** (1960a) 561.
90. P. J. Garrahan and I. M. Glynn, *J. Physiol. (Lond.)*, **192** (1967a) 159.
91. P. J. Garrahan and I. M. Glynn, *J. Physiol. (Lond.)*, **192** (1967b) 175.
92. P. J. Garrahan and I. M. Glynn, *J. Physiol. (Lond.)*, **192** (1967c) 189.
93. P. F. Baker, M. P. Blaustein, R. D. Keynes, J. Manil, T. I. Shaw and R. A. Steinhardt, *J. Physiol. (Lond.)*, **200** (1969) 459.
94. R. A. Sjodin and L. A. Beaugé, *J. Gen. Physiol.*, **51**, No. 5, Part 2 (1968) 152.
95. F. J. Brinley and L. J. Mullins, *J. Gen. Physiol.* **52** (1968) 181.
96. P. F. Baker, *Biochim. Biophys. Acta*, **88** (1964) 458.
97. A. F. Lant, R. N. Priestland and R. Whittam, *J. Physiol. (Lond.)*, **207** (1970) 291.
98. P. C. Caldwell, A. L. Hodgkin, R. D. Keynes and T. I. Shaw, *J. Physiol. (Lond.)* **152** (1960b) 591.
99. P. de Weer, *J. Gen. Physiol.*, **56** (1970) 583.
100. I. M. Glynn and J. F. Hoffman, *J. Physiol. (Lond.)*, **218** (1971) 239.
101. S. Nahajima and K. Tabakaski, *J. Physiol. (Lond.)*, **187** (1966) 105.
102. T. Thomas, *J. Physiol. (Lond.)*, **201** (1969) 495.
103. P. C. Caldwell, *Physiol. Rev.*, **48** (1968) 1.
104. J. F. Hoffman and F. M. Kregenow, *Ann. N.Y. Acad. Sci.*, **137** (1966) 566.
105. J. D. Judah, K. Ahmed, A. E. M. Maclean, *Biochim. Biophys. Acta*, **65** (1962) 472.
106. M. Fujita, T. Nakao, N. Mizuno, K. Nagano and M. Nakao, *Biochim. Biophys. Acta*, **117** (1966) 42.
107. B. Formby and J. Clausen, *Z. Physiol. Chem.*, **349** (1968) 909.
108. H. Yoshida, K. Nagai, T. Ohashi and Y. Nakagawa, *Biochim. Biophys. Acta*, **179** (1969) 178.
109. P. J. Garrahan, M. I. Pouchan and F. Rega, *J. Membrane Biol.*, **3** (1970) 14.
110. F. Rega, P. J. Garrahan and M. I. Pouchan, *J. Membrane Biol.* **3** (1970) 26.
111. A. Askari and G. Koyal, *Biochem. Biophys. Acta*, **225** (1971) 20.
112. G. Koyal, S. N. Rao and A. Askari, *Biochim. Biophys. Acta*, **225** (1971) 11.
113. A. Askari and S. N. Rao, *Biochim. Biophys. Acta*, **241** (1971) 75.