

*Hypothesis*Is Ca^{2+} -ATPase a water pump?

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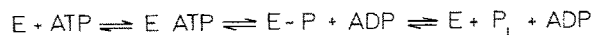
The mechanism of free energy coupling in active transport is discussed with special reference to the sarcoplasmic reticulum Ca^{2+} -ATPase. In the current working schemes for cation transport ATPases, free energy transduction is nearly always based on enzyme conformational changes. The principal objective of the present article is to examine whether recent experimental results on Ca^{2+} -ATPase may in fact be better explained by assuming the existence of a direct chemiosmotic process. In the scheme proposed, free energy transduction between ATP and calcium is based on a transfer of solvation water between the acyl-phosphate bond and the bound calcium ions.

<i>Transport ATPase</i>	<i>Energy transduction</i>	<i>Water</i>	<i>Sarcoplasmic reticulum</i>
	<i>Calcium</i>	<i>Phosphorylation</i>	

1. INTRODUCTION

This paper deals with the mechanism of free energy transduction coupled to vectorial processes in transport ATPases. It is concerned in particular with the Ca^{2+} -ATPase of sarcoplasmic reticulum, but this discussion may be significant for other transport ATPases, especially for those in which a phosphorylated intermediate has been determined.

The sarcoplasmic reticulum Ca^{2+} -ATPase catalyses the two following reactions (1,2):



More than two decades of work indicate that these two reactions are tightly coupled proceeding synchronously step by step. This coupling enables the Ca^{2+} -ATPase to convert chemical into osmotic energy with high efficiency. The theoretical difficulties in designing realistic working schemes for transport enzymes are different from those for

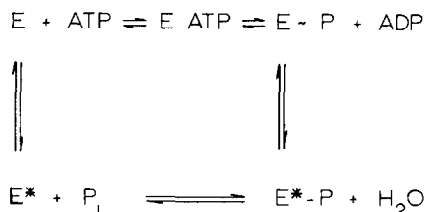
typical biochemical reactions. Firstly because only free energy is apparently transferred between the energy-donating (1) and the energy-accepting reaction (2); secondly because it is not simple to imagine how the ion can be transported over a large distance through the membrane. Consequently there has been a general tendency to invoke completely indirect coupling between the reactions (1) and (2). In general it has been proposed that the phosphate transfer reaction and the ion transport occur in physically separated and specialized domains of the enzyme and that one key conformational transition of the protein provides the link between the events occurring within these two domains [1-4].

In the first part of this article, recent experimental results on Ca^{2+} -ATPase will be briefly discussed. It will be shown that trying to use these data within the general framework of a completely indirect conformational coupling leads to an increasing complexity of the reaction scheme. This type of scheme has to be modified to such a point that its meaning may be almost completely lost.

In the second part of this article a mechanism will be presented and discussed in which the free energy transduction process is not associated with one particular conformational change of the enzyme. It will be shown that free energy transduction and vectorial processes in transport ATPases may readily be explained by the chemical and solvation properties of the substrates. This type of mechanism is very close to that advocated in [5,6].

2. CONFORMATIONAL COUPLING

The central question on the mechanism of transport ATPases concerns the nature of the coupling between osmotic and chemical forces. The most precise schemes used so far for plasma membrane transport ATPases are of the family of so-called mechanochemical schemes where a major conformational change is supposed to cause the ion binding sites to be alternatively exposed to one or the other side of the membrane with a synchronous change in reactivity of the acyl-phosphate intermediate. This key role allocated to the enzyme conformation is the basis of the $E \rightleftharpoons E^*$ scheme of sarcoplasmic reticulum Ca^{2+} -ATPase [1,2] and of the closely related $E1 \rightleftharpoons E2$ scheme of $(\text{Na}^+, \text{K}^+)\text{-ATPase}$ [3,4]. The basic proposals of this two-state scheme are summarized in scheme 3 for the Ca^{2+} -ATPase:



The $E \rightleftharpoons E^*$ isomerization of Ca^{2+} -ATPase was indeed detected by following the intrinsic fluorescence of the protein [7] and it was found that, as proposed in [2], the equilibrium between the two conformers is dependent on cytoplasmic calcium concentration. Many other studies then followed, indicating that nearly all other known substrates of Ca^{2+} -ATPase (ATP, ADP, P_i and Mg^{2+}) have a significant effect on the protein conformation. It is beyond the scope of the present paper to report these results in detail and a recent review may be found in [8]. The most important contribution of the fluorescence techniques has been to permit accurate kinetic measurements not feasible with other biochemical techniques. In recent years very precise fluorescence experiments have been designed to investigate the reaction scheme of Ca^{2+} -ATPase [8]. In many aspects, the results do not support the simple two-state scheme as described in table 1.

The most important results reported so far are:

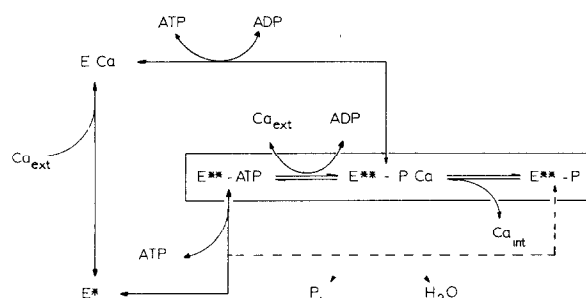
- (i) The detailed sequence of calcium binding implies that, at least for the unphosphorylated enzyme, one half of the high affinity calcium binding sites are always rapidly accessible from the cytoplasmic side of the vesicles [9]. This observation clearly conflicts with the situation described in table 1.
- (ii) The $E \sim \text{P} \rightarrow E^* \sim \text{P}$ conversion is not associated with reversal of the conformational change observed during the $E^* \rightarrow E$ transition.
- (iii) A third conformational state E^{**} should be introduced which is stabilized by either ATP binding or phosphoenzyme formation (independently of the 'energy content' of the phosphoenzyme formed) [10].

A consequence of this is that, during turn-over and in the presence of moderate ATP concentra-

Table 1

Conformation	Calcium sites	Phosphoenzyme
E	High affinity accessible from the cytoplasm	$E \sim \text{P}$ 'high energy' reacting with ADP
E^*	Low affinity accessible from the inner side	$E^* \sim \text{P}$ 'low energy' reacting with water

tions, the enzyme undergoes no sizeable intrinsic fluorescence change and is stabilized in the intermediate state E^{**} with very low probability for the existence of the other two states E and E^* [10]. This situation is summarized in scheme 4:



We recently proposed [8] that these results could be reconciled with the classical $E \rightleftharpoons E^*$ scheme by assuming that the active ATPase is a dimer and that during turn-over the enzymatic state E^{**} which is accumulated corresponds in fact to the hybrid intermediate $E \cdot E^*$. The observation that the conformation of the enzyme is apparently stable during turn-over is in favor of a model where the two subunits are describing the $E \rightleftharpoons E^*$ cycle out-of-phase (flip-flop). The soundness of such a model is presently not easy to test. Moreover other independent proofs for the existence of a functional dimeric association are lacking and also some properties of the intermediate E^{**} state are unique and as such can in no simple way be explained by hybrid association of E and E^* . These properties are:

- (i) In the intermediate phosphorylated state: $E^{**} \sim P$, the ion binding sites are occluded (i.e., inaccessible from both sides of the membrane) [11];
- (ii) Changes in the reactivity of the acyl-phosphate intermediate ($E^{**} \sim P$ to $E^{**} - P$) appear to be linked to local changes in hydration of the active site [12,13]. These changes have been detected with a fluorescent analog of ATP and occur only when the enzyme is stabilized in the E^{**} conformation.

It is not clear how further amendments of the $E \rightleftharpoons E^*$ scheme, exclusively based on conformational coupling, could take into account these last data. These considerations are obviously not sufficient to definitively reject the conformational

coupling hypothesis. In what follows, however, an alternative possibility will be investigated where, as advocated in [5], the enzyme conformational changes are not mechanically coupled to the ion translocation process but are simply a way for the enzyme to optimize interactions between the substrates bound in the catalytic site. In this hypothesis the stabilization of the intermediate configuration adopted by the enzyme during turn-over would be analogous to the accumulation of the transition state in more classical enzymatic reactions. The vectorial nature of the transport process should be, in this hypothesis, a direct consequence of the chemical properties of the reactants or of the geometry of the active site.

3. CHEMIOSMOTIC COUPLING

3.1. The phosphate transfer reaction

During the turn-over cycle of the Ca^{2+} -ATPase enzyme the terminal phosphate of ATP is transferred to water through a relatively well defined pathway (see scheme 3). (For simplicity the nomenclature in [1] will be used.) The phosphate bond in the $E \sim P$ state is referred to as 'high energy' because of its ability to react reversibly with ADP to form ATP. This is not the case for the $E^* - P$ or 'low energy' intermediate which can only react with water to liberate P_i . It is therefore thought that it is during the $E \sim P$ to $E^* - P$ transition that the free energy of the phosphate bond is lost and utilized to raise the chemical potential of the calcium ions bound to the protein.

Authors in [12] proposed that the apparent energy content of the acyl-phosphate bond is dependent on the water activity within the active site. This hypothesis is based on the work in [14] and [15] in which it was calculated that solvation energies of the reactants are the predominant factors determining the free energy content of energy-rich compounds.

We have recently confirmed the hypothesis in [12] and observed that the $E \sim P$ to $E^* - P$ transition and phosphorylation by P_i ($E^* + P_i \rightleftharpoons E^* - P$) was associated with a significant reduction in polarity of the active site accompanied by liberation of a large number of water molecules [13]. A consequence of this process is that the 'low energy' phosphate bond $E^* - P$ is likely to be completely isolated from solvent water. According to the

calculations in [15] this leads to the formation of a stable 'low energy' bond. The experiments described in [13] have an essential role in the present discussion since the following facts were observed:

- (i) The change in hydration (or polarity) undergone by the catalytic site during the $E \sim P$ to $E^* - P$ transition which is the heart of the energy transduction process, according to the discussion above, is not associated with a significant conformation change of the protein [8,10];
- (ii) The largest conformational change so far observed by following the intrinsic fluorescence of the protein ($E^* + Ca \rightleftharpoons E \cdot Ca$) has no effect on the polarity of the active site;
- (iii) The change in hydration described in (i) is apparently associated with binding or dissociation of Ca^{2+} from internal low affinity sites ($K_d = 10$ mM).

The first two points reinforce the idea developed at the end of the preceding section that there could be no direct link between conformational changes and ion transport.

The last point leads to the next question: could the change in hydration of the phosphorylation site be triggered by direct binding or dissociation of Ca^{2+} and Mg^{2+} within the catalytic site? The likelihood of such a mechanism will now be examined.

3.2. Calcium binding

Calcium ions exhibit 3 main forms of association with the Ca^{2+} -ATPase enzyme: a strong association to its cytoplasmic sites, an occluded form where Ca^{2+} can be only very slowly exchanged and a weak association to inwards accessible sites. What needs to be understood in chemical terms is the precise meaning of these different associations.

3.2.1. Ca^{2+} and water

It is known that metal ions in water form structured complexes with hydration water; complexes formed by calcium are relatively labile so that hydration numbers varying from 4 to 9 have been reported by various techniques [16–19]. In [19], using neutron diffraction, authors reported that the aquo-calcium complex is most probably hexa-

coordinated as reported for magnesium and other metal ions [18].

3.2.2. High affinity calcium sites

The high affinity calcium sites of Ca^{2+} -ATPase are very selective for Ca^{2+} with an association constant around $3 \times 10^6 M^{-1}$. The process of high affinity binding to free ligands in water [20], to liposoluble antibiotics [21] and to calcium binding proteins whose crystal structure is known [22] is relatively well understood. As for the formation of complex metal ions [23,24] it involves the substitution of coordinated water molecules of the octahedral aquocalcium, $[Ca(H_2O)_6]^{2+}$, by stronger coordination bonds with chelating groups of the ligand. Various coordination numbers have been reported to occur after association [22], but apparently high affinity binding implies a nearly complete dehydration of Ca^{2+} . It is very likely that binding to the high affinity sites of Ca^{2+} -ATPase proceeds via a similar process. This idea is further substantiated in [25] where the electron spin relaxation of gadolinium ions bound to the high affinity sites of Ca^{2+} -ATPase were measured. They suggested that the bound gadolinium ions are much less hydrated than in solution and that the binding sites have a reduced accessibility of solvent water.

3.2.3. Occluded calcium

Calcium ions bound to the external high affinity sites become very slowly exchangeable after phosphorylation by ATP [11,26–29] ($K_{exch.} < 0.1 s^{-1}$). A third occluded metal ion binding site was also found normally occupied by Mg^{2+} which, however, can be replaced by calcium when Ca -ATP is used as a substrate in magnesium-free medium [11]. When Mg -ATP or Ca -ATP are added to an enzyme saturated with calcium the rate of metal ion occlusion is extremely fast and occurs at the same rate as that of phosphoenzyme formation, within the first millisecond of ATP addition [11,26]. On the other hand, phosphorylation can only occur when the 3 metal ion binding sites are occupied (at least 2 of them with calcium). The catalytic action of divalent cations on phosphoryl transfer is a well documented subject which has been studied in detail in enzymes and model systems [30–33]. It has also been demonstrated that phosphoryl transfer reactions in Ca^{2+} -ATPase proceed via an in-line $Sn2$ displacement reaction

[34] with the probable transient formation of a pentavalent transition state. As proposed in [33], this process may be catalyzed by the presence of electrophilic groups whose role is to stabilize the transition state and lower the activation energy of the phosphoryl transfer reaction. These electrophilic centers may be acidic residues but also metal cations bound by other coordination bonds to the protein.

It is proposed here that the 3 metal ions catalyzing the phosphorylation of Ca^{2+} -ATPase are bound close to the phosphorylation site in such positions that they may form a good template for the 3 planar coordinations of the pentavalent intermediate. After phosphoenzyme formation and release of ADP the 3 divalent cations would remain strongly associated with the phosphate so that they become much less exchangeable (occluded). A close association between magnesium, monovalent cations and phosphate was also proposed for the $(\text{Na}^+, \text{K}^+)\text{-ATPase}$ by authors in [32,35–38] who measured, by EPR and NMR, that 55 Mn, 205 Tl, 7 Li and 31 P were confined within less than 10 Å in the active site of this enzyme.

3.2.4. Low affinity calcium sites

The existence of inner low affinity calcium sites has been postulated to account for the effect of millimolar internal calcium concentrations on the $\text{E}^*\text{-P}$ to $\text{E}\text{-P}$ conversion [2]. The topology of the enzyme implies that, to reach the active site of the protein from the inner side of the vesicles, the ions have to move through the membrane over a relatively long distance (40–50 Å) most probably through some kind of channel formed within the hydrophobic tail of the Ca^{2+} -ATPase. Indeed, it has been reported that membrane-bound tryptic fragments of Ca^{2+} -ATPase can form calcium channels with low specificity compared to other cations [39]. The low apparent affinity for the effect of internal calcium and the low specificity of the channel most probably indicate that the mechanism of association between internal calcium and the Ca^{2+} -ATPase does not involve first sphere coordination bonds. In other words this means that during their travel through the channel formed by the protein, Ca^{2+} is hydrated and able to form only outer sphere weak bonds with the amino acid residues. It is clear that a more specific coordination bond would have the effect

of strongly reducing the mobility of the transported ion during its transit. The fact that water molecules and ions have to comigrate through protein or protein-like channels is not new, and has been studied in detail for gramicidin and for the potassium channel of sarcoplasmic reticulum [40–42]. Six water molecules in the first case and 2–3 in the latter have been reported to be associated with each single transported monovalent cation. No such data exists for calcium channels but the higher charge of the calcium ion makes this process even more plausible.

3.3. Water as a source of chemiosmotic coupling

After the discussion above and the work in [2,12] and [13], it has become clear that any reaction scheme of Ca^{2+} -ATPase should now be rewritten to include explicitly the interaction of both soluble and bound substrates with water. As discussed above the decrease in phosphoenzyme-free energy is probably associated with dehydration of the acyl-phosphate bond. It is proposed here that the lost water molecules are transferred to the hydration shell of the bound Ca^{2+} , resulting in a modification of the bonds formed by the transported ion and a lowering of its association constant with the enzyme. It is important to understand that this structural modification of the transported species may well be a way for the enzyme to create a vectorial reaction. This will happen if the transit through the high affinity cytoplasmic access is only allowed for the dehydrated form of calcium and if the only choice for the rehydrated ion to leave the active site is to pass through an ion channel opening to the inner side of the vesicle.

The following working scheme (5) is therefore proposed (p.19).

Species in square brackets represent solvated substrates. For clarity, phosphate transfer and calcium translocation reactions have been represented separately. Binding of the substrates is uncoupled so that association of $[\text{ATP}]$, $[\text{P}_i]$, $[\text{Ca}]_{\text{out}}^{2+}$ and $[\text{Ca}]_{\text{in}}^{2+}$ can occur randomly, inducing the conformational changes observed by measuring the protein fluorescence. Coupling between the two reactions is represented on this scheme by the steps 1 and 2. Step 1 represents the coupling bet-

ween phosphoenzyme formation and calcium occlusion, and step 2 the coupling between the 'high energy' to 'low energy' phosphate bond conversion and calcium translocation. By doing this, it is not meant that free energy transduction is specifically localized within this latter step. Indeed the fact that phosphate and calcium ions are not exchangeable after step 1 prevents an evaluation of their individual chemical energies. On the other hand, transfer of solvation water has been arbitrarily located in step 2 although sharing of water molecules may have occurred before this step.

One of the most remarkable properties of an ion pump following the scheme proposed above is that it also behaves as a water pump. Eighteen water molecules, at least, have been reported to enter the catalytic site during the rehydration process associated with P_i release [13]. A large fraction of these is probably transported through the membrane at the end of the next cycle in association with Ca^{2+} , this pathway of water is represented as a heavy line in scheme 5. The dashed lines represent the parts of the cycle where the calcium binding and phosphorylation sites experience low water activity.

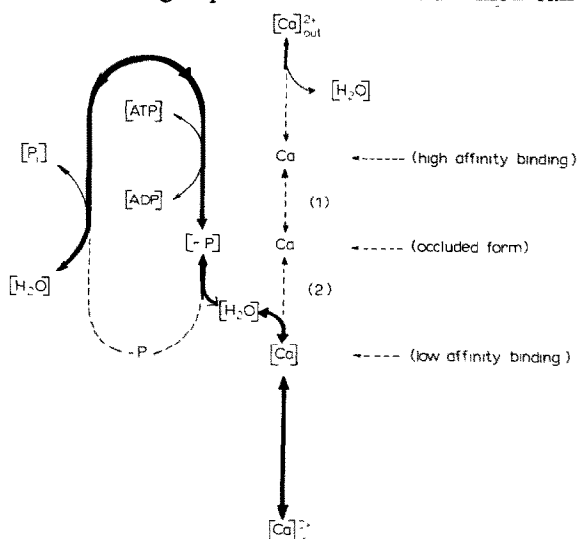
4. CONCLUSIONS

The ideas developed in the preceding section are at present mainly hypothetical. It is, however, the objective of this article to propose a consistent and precise chemiosmotic scheme which can account for the existing experimental data and which can

be tested in future experimental work. As a working hypothesis, it is proposed that free energy transduction between ATP and calcium is based on a transfer of solvation water between the acyl-phosphate intermediate and calcium while bound in the active site of the enzyme, without implying an obligatory conformational transition of the protein. To make this system feasible, the enzyme must have the following properties:

- (i) The Ca^{2+} -ATPase enzyme needs to possess an active site where calcium, magnesium, phosphate and water can interact at close range. The conformational changes observed in earlier work only serve for the enzyme to bind specifically the substrates and to optimize their interactions;
- (ii) The active site is accessible to calcium via two asymmetric 'channels': the first access opens to the cytoplasmic side of the enzyme and contains the high affinity specific calcium sites (selectivity filter), access via this route implying dehydration of Ca^{2+} ; the second access is an ion channel probably buried within the tail of the enzyme and opening to the inner side of the vesicle. To travel through this channel Ca^{2+} should be rehydrated at the expense of the acyl-phosphate intermediate.

A final remark concerns the possibility that Ca^{2+} -ATPase may indeed exchange calcium for one or more cations. This point is an open debate which has been deliberately neglected in this article. The observation that potassium ions activate dephosphorylation [2] and a recent report showing that Ca^{2+} -ATPase catalyzes a monovalent cation exchange [43] make the existence of an inverse potassium transport plausible. This process may be included in scheme 5 by assuming that dissociation of phosphate and rehydration of the active site after calcium transport can be accelerated by an entry of hydrated potassium ions through the ion channel. This is obviously different from a re-entry of hydrated calcium which would simply have the effect of promoting a reverse $-P$ to $[\sim P]$ transition; this ion selectivity is plausible since potassium ions have an hydration number and an hydration radius different from that of calcium [16] and may be unable to establish the correct interactions with phosphate. It is clear that this kind of mechanism may serve as a model for other transport ATPases and particularly for (Na^+, K^+) -ATPase.



REFERENCES

- [1] Carvalho, M.G.C., deSouza, D.G. and deMeis, L. (1976) *J. Biol. Chem.* 251, 3629–3626.
- [2] deMeis, L. (1981) in: *The Sarcoplasmic Reticulum* (Bittar, E.E. ed) Wiley, New York.
- [3] Post, R.L., Hegevary, C. and Kume, C. (1972) *J. Biol. Chem.* 247, 6530–6540.
- [4] Albers, J.W. (1976) in: *The Enzymes of Biological Membranes* (Martonosi, A.N. ed) vol.3, pp.283–301.
- [5] Mitchell, P. (1979) *Eur. J. Biochem.* 95, 1–20.
- [6] Mitchell, P. and Koppenol, W.H. (1982) *Ann. NY Acad. Sci.* 402, 584–601.
- [7] Dupont, Y. (1976) *Biochem. Biophys. Res. Commun.* 71, 544–550.
- [8] Dupont, Y., Bennett, N., Pougeois, R. and Lacapere, J.J. (1983) in: *Structure and Function of Sarcoplasmic Reticulum* (Tonomura, Y. and Fleisher, S. eds) Academic Press, New York.
- [9] Dupont, Y. (1982) *Biochim. Biophys. Acta* 688, 75–87.
- [10] Dupont, Y., Bennett, N. and Lacapere, J.J. (1982) *Ann. NY Acad. Sci.* 402, 569–572.
- [11] Dupont, Y. (1980) *Eur. J. Biochem.* 109, 231–238.
- [12] deMeis, L., Martins, O.B. and Alves, E.W. (1980) *Biochemistry* 19, 4252–4261.
- [13] Dupont, Y. and Pougeois, R. (1983) *FEBS Lett.* 156, 93–98.
- [14] George, P., Witonsky, R.J., Trachtman, M., Wu, C., Dorwart, W., Richman, W., Richman, L., Shurayh, F. and Lentz, B. (1970) *Biochim. Biophys. Acta* 223, 1–15.
- [15] Hayes, D.M., Kenyon, G.L. and Kollman, P.A. (1978) *J. Am. Chem. Soc.* 100, 4331–4340.
- [16] Marcus, Y. (1977) in: *Introduction to Liquid State Chemistry*, pp.250–252, Wiley, New York.
- [17] Swift, J.J. and Sayre, W.G. (1966) *J. Am. Chem. Soc.* 44, 3567–3574.
- [18] Matwiyoff, N.A. and Taube, H. (1968) *J. Am. Chem. Soc.* 90, 2796–2800.
- [19] Cummings, S., Enderby, J.E. and Howe, R.A. (1980) *J. Phys. C: Solid St. Phys.* 13, 1–18.
- [20] Williams, R.J.P. (1980) in: *Calcium Binding Proteins* (Siegel, F.L. et al. eds) Elsevier, Amsterdam, New York.
- [21] Smith, G.B. and Duax, W.L. (1976) *J. Am. Chem. Soc.* 98, 1578–1580.
- [22] Kretsinger, R.H. (1976) *Annu. Rev. Biochem.* 45, 239–266.
- [23] Eigen, M. and Tamm (1962) *Z. Electrochem.* 66, 107–121.
- [24] McAuley, A. and Hill, J. (1969) *Quart. Rev.* 23, 18–36.
- [25] Stephens, E.M. and Grisham, C.M. (1979) *Biochemistry* 18, 4876–4885.
- [26] Verjovski-Almeida, S., Kurzmack, M. and Inesi, G. (1978) *Biochemistry* 17, 5006–5013.
- [27] Takakuwa, Y. and Kanazawa, T. (1979) *Biochem. Biophys. Res. Commun.* 88, 1209–1216.
- [28] Takisawa, H. and Makinose, M. (1981) *Nature* 290, 270–273.
- [29] Serpesu, E.H., Kirch, U. and Schoner, W. (1982) *Eur. J. Biochem.* 122, 347–354.
- [30] Tetas, M. and Loewenstein, J.M. (1963) *J. Biol. Chem.* 2, 350–357.
- [31] Johnson, R.A. and Selzer, S. (1973) *J. Am. Chem. Soc.* 95, 936–939.
- [32] Mildvan, A.S. and Grisham, C.M. (1974) *Structure and Bonding* 20, 1–21.
- [33] Knowles, J.R. (1980) *Annu. Rev. Biochem.* 49, 877–919.
- [34] Webb, M.R. and Trentham, D.R. (1981) *J. Biol. Chem.* 256, 4884–4887.
- [35] Grisham, C.M. and Mildvan, A.S. (1974) *J. Biol. Chem.* 249, 3187–3197.
- [36] Grisham, C.M., Gupta, R.K., Barnett, R.E. and Mildvan, A.S. (1974) *J. Biol. Chem.* 249, 6738–6744.
- [37] Grisham, C.M. and Hutton, W.C. (1978) *Biochem. Biophys. Res. Commun.* 81, 1406–1411.
- [38] O'Connor, S.E. and Grisham, C.M. (1980) *FEBS Lett.* 118, 303–307.
- [39] Herrmann, T.R. and Shamoo, A.E. (1982) in: *Membranes and Transport* (Martonosi, A.N. ed) vol.1, 579–584.
- [40] Levitt, D.G., Elias, S.R. and Hautman, J.M. (1978) *Biochim. Biophys. Acta* 512, 436–451.
- [41] Finkelstein, A. and Andersen, O.S. (1981) *J. Membr. Biol.* 59, 155–171.
- [42] Miller, C. (1982) *Biophys. J.* 38, 227–230.
- [43] Dupont, Y. and Bennett, N. (1983) in: *Structure and Function of Sarcoplasmic Reticulum* (Tonomura, Y. and Fleisher, S. eds) Academic Press, New York.