We believe that the above data can be most easily explained in terms of a four-subunit enzyme, whether the source of the lactate dehydrogenase was beef heart or dogfish muscle. The observed splitting was most likely due to the presence of some contaminant in one lot of the commercial guanidine hydrochloride used. Such observations of splitting in concentrated guanidine solutions have been reported for pig and beef heart lactate dehydrogenase, as well as for β-lactoglobulin and bovine serum albumin (Jaenicke, 1969) and for spleen acid deoxyribonuclease (Townend and Bernardi, 1971). Since both β-lactoglobulin and bovine serum albumin are known to have single polypeptide chains, the splitting of covalent bonds would be obligatory in these two cases. Such splitting is the most likely cause of the molecular weight decreases observed with beef heart lactate dehydrogenase in the present study.

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Phosphorylation of the Membranous Protein of the Sarcoplasmic Reticulum Inhibition by Na^+ and K^+†

Leopoldo de Meis

ABSTRACT: Ca^{2+} activates the transfer of the γ-phosphate of ATP to a protein of the sarcoplasmic reticulum. Na^+ and K^+ strongly inhibit this reaction by competing with Ca^{2+} for its binding site. The degree of inhibition varies with ATP concentration and temperature. It is proposed that the binding of ATP to the membrane produces a conformational change of the Ca^{2+} binding site resulting in a modification of its specificity.

Fragmented sarcoplasmic reticulum isolated from skeletal muscle retains a highly efficient ATP-dependent Ca^{2+} transport system (Hasselbach and Makinose, 1961; Hasselbach, 1964). The active Ca^{2+} transport is mediated by a membrane-bound ATPase which is highly sensitive to change in free Ca^{2+} concentration on either side of the membrane. In the process of ATP hydrolysis, the γ-phosphate of ATP is covalently bound to a membrane protein (E). This phosphoprotein (E∼P) represents an intermediate product in the sequence of reactions leading to Ca^{2+} transport and phosphate

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liberation. The following sequence has therefore been proposed (Makinose, 1969; Friedman and Makinose, 1970; Inesi *et al.*, 1970)

$$ATP + E \rightleftharpoons E\sim P + ADP \quad (1)$$

$$E\sim P \longrightarrow E + P_i \quad (2)$$

Previous reports (de Meis, 1969, 1970, 1971; de Meis and Hasselbach, 1971) have shown that when ATP concentration in the assay medium is between 2 and 10 μM, Na^+ and K^+ inhibit both Ca^{2+} uptake and ATPase activity of the sarcoplasmic vesicles. When the ATP concentration was raised to a range of 0.1–4.0 mM, inhibition was no longer observed. In this report, the effect of alkali ions on the E∼P formation was studied both at 0 and 37° using varying ATP concentrations.

Material and Methods

Sarcoplasmic reticulum vesicles were prepared from rabbit skeletal muscle as previously described (de Meis and Hasselbach, 1971).

[γ-^{32}P]ATP. This was prepared by a slightly modified method of Glynn and Chapell (1964). The reaction mixture composition was 6 mM $MgCl_2$, 2 mM cysteine, 1 mM 3-phosphoglycerate as the trycyclohexylammonium salt, 4 mM ATP, 0.4 mM NADH, 1 mM EGTA,[1] 50 mM Tris-HCl buffer, pH 8.1, and 20–50 mCi of "carrier-free" ^{32}P. The ^{32}P solution was adjusted to pH 7.0 before addition to the reaction mixture.

The following were added to 13 ml of reaction mixture: 0.15 ml of 2-mercaptoethanol; 200 μg of muscle D-glyceraldehyde 3-phosphate:NAD oxireductase (phosphorylating) (EC 1.2.1.12) diluted in 0.5 ml of water and 100 μg of ATP:3-phospho-D-glycerate 1-phosphotransferase (EC 2.7.2.3) diluted in 0.5 ml of water. After incubation at room temperature for 1 hr, the reaction was stopped by heating the mixture in boiling water for 1 min. The mixture was cooled immediately in ice-cold water and filtered. The filter paper was washed with deionized water until the mixture was diluted to 50 ml. Labeled ATP was separated by a column of Dowex 1 resin as described by Glynn and Chapell (1964). Usually, 85–90% of the ^{32}P was recovered in the form of [γ-^{32}P]ATP.

[^{32}P]Phosphocreatine. Twelve ml of a solution containing 50 mM buffer glycine–KOH, pH 9.0, 4 mM $MgSO_4$, 5 mM creatine, and 0.2 mM [γ-^{32}P]ATP was combined with 0.6 mg of ATP:creatine phosphotransferase (EC 2.7.3.2). Volumes of 0.1 ml of a 24 mM ATP solution (nonradioactive) were added after 45 and 90 min of incubation at 30°. After another 1-hr incubation, the mixture was stored at −20° until use. Before use, the pH of the solution was adjusted to 7.0 with HCl and diluted with nonradioactive phosphocreatine to the desired concentration.

Standard Incubation Medium. Unless otherwise stated, the incubation medium consisted of 10 mM Tris-maleate buffer, pH 7.0, 4 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.26 mM EGTA, and the specified concentrations of [^{32}P]ATP and NaCl or KCl. The pH of the final mixture was 6.85. The free Ca^{2+} concentration for the $CaCl_2$ and EGTA concentration given was calculated using the dissociation constant 4×10^{-6} M (de Meis and Hasselbach, 1971). Accordingly, for the medium described, the Ca^{2+} concentration was 10.6 μM.

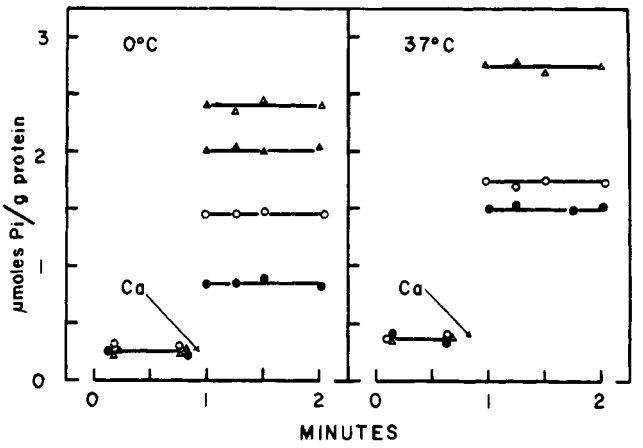

FIGURE 1: Time course of E∼P formation and its dependence on Ca^{2+} and Mg^{2+}. [^{32}P]ATP concentration was 4 μM. Other additions were as described under Methods with 0.26 mM EGTA but without $CaCl_2$. The arrow points to the addition of $CaCl_2$, final concentration of 0.20 mM. The calculated free Ca^{2+} concentration was 10.6 μM. Other experimental conditions were as described under Methods. Right: Reaction carried out at 0°. Final volume of the assay medium was 40 ml. The sarcoplasmic vesicles protein concentration was 50 μg/ml. —△— Control medium; —▲— control medium plus 40 mM LiCl; —○— control medium plus 40 mM KCl; —●— control medium plus 40 mM NaCl. Left: The reaction was carried out at 37°. The final volume of the assay medium was 8 ml, containing 1 mM [^{32}P]phosphocreatine and 25 μg/ml of ATP:creatine phosphotransferase. The sarcoplasmic vesicles protein concentration was 250 μg per ml. —△— Control medium; —○— control medium plus 40 mM potassium acetate; —●— control medium plus 40 mM sodium acetate.

The assay medium to which no monovalent cation was added, was referred to as control medium. The amount of sarcoplasmic vesicles added was 2 mg of protein. When the ATP concentration was 2 mM, the final volume was 4 ml. When the ATP concentration varied between 4 and 10 μM and the reaction was performed at 37°, the final volume was 8 ml. In these low ATP experiments 1 mM [^{32}P]phosphocreatine and 25 μg/ml of ATP:creatine phosphotransferase were included in the assay medium. In control experiments, the ATPase activity of the vesicles was measured as a function of the microsomal protein concentration as previously described (de Meis, 1971). A straight line was obtained in the range of protein concentration of 0–0.5 mg/ml. When the reaction was performed at 0° with these low ATP concentrations, the ATP regenerative system was omitted, and the final volume was 40 ml. The aim of this procedure was to increase the total amount of ATP in order to avoid substrate exhaustion. Figure 1 shows a typical experiment, in which the amount of ^{32}P incorporated into the vesicular protein was measured as a function of the incubation time. When Ca^{2+} was added to the control medium, the amount of E∼P increased immediately to 2.4 μmoles per gram of protein. This amount persisted when the vesicles were incubated for periods of up to 1 min. For longer incubation intervals, the amount of E∼P decreased progressively due to substrate exhaustion. In control experiments, the ATPase activity was estimated from the amount of ^{32}P present in the charcoal-treated supernatant (Post *et al.*, 1965). After 30-sec incubation in presence of Ca^{2+}, less than 10% of the ATP was hydrolyzed. Thus, in the following experiments, the reaction was always stopped after 10 sec of incubation.

[1] Abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

TABLE I: Effect of Temperature and ATP Concentration on the Ca^{2+} Transport, ATPase Activity, and E∼P Formation.[a]

ATP Concentration (M)	Temperature (°C)	E∼P_i (μmoles of P_i/g of protein)	Ca^{2+} Uptake (μmoles of Ca^{2+}/mg of protein min^{-1})	ATPase Activity (μmoles of P_i/mg of protein min^{-1})
4×10^{-6}	0	2.32 ± 0.29 (9)	>0.01 (9)	>0.01 (9)
	37	1.96 ± 0.33 (6)	0.90 ± 0.1 (6)	0.41 ± 0.10 (6)
2×10^{-3}	0	1.91 ± 0.17 (4)	0.04 ± 0.01 (4)	0.03 ± 0.01 (4)
	37	1.81 ± 0.15 (4)	3.50 ± 0.20 (4)	1.80 ± 0.20 (4)

[a] The assay medium composition and the experimental conditions were as described under Methods. The values represent the average ± std error of the number of experiments indicated in parentheses.

Standard Assay. The reaction was started by the addition the sarcoplasmic reticulum vesicles and stopped by either perchloric acid or trichloroacetic acid. When the volume of the assay medium was 4 ml or 8 ml, it was injected into 20 ml of ice-cold solution of 125 mM perchloric acid containing 2 mM orthophosphate. When the volume of the assay medium was 40 ml, 2 ml of an ice-cold solution of trichloroacetic acid at 100% (w/v) containing 20 mM orthophosphate was added during vigorous stirring at 0°. The suspensions were centrifuged in the cold at 5000*g* for 10 min. The protein pellet was washed five times in ice-cold 125 mM perchloric acid solution containing 2 mM orthophosphate. In the second washing, the pellet was initially resuspended in 0.5 ml of 10 mM ATP and then the perchloric acid solution was added. After the washings, the pellet was resuspended in 1 ml of a solution containing 0.1 N NaOH, 2% Na_2CO_3, and 1 mM orthophosphate. The pellet was dissolved by heating the suspension in boiling water for 30 min. After cooling, an aliquot was dried in a planchette and counted in a Nuclear-Chicago gas flow counter. Another aliquot was used for protein determination by the method of Lowry *et al.* (1951).

Ca^{2+} Uptake and ATPase Activity. These were assayed as previously described (de Meis, 1969).

Results

Mg^{2+} and Ca^{2+} Dependency of the P_i Transfer Reaction. Two different ATPase activities can be distinguished in skeletal muscle microsomes. One requires only Mg^{2+} for its activation and has been referred to in the literature as Mg^{2+}-dependent ATPase. The second requires in addition Ca^{2+} for its full activation and has been referred to as Ca^{2+}-activated ATPase (Hasselbach, 1964). Several reports have shown that the Ca^{2+}-activated ATPase is intimately associated with the Ca^{2+} transport in muscle microsomes. Accordingly, it has been shown that two different levels of E∼P are obtainable, depending on the divalent cation added to the medium (Makinose, 1969). Figure 1 shows a typical experiment. In the absence of Ca^{2+}, 0.2–0.4 μmole of P_i was incorporated per gram of vesicular protein. When Ca^{2+} was added to the control medium, the amount of E∼P increased in less than 5 sec to the range of 1.8–3.0 μmoles per gram of protein. This agrees with the data reported in the literature (Makinose, 1969; Inesi *et al.*, 1970). Essentially the same degree of Mg^{2+}-dependent phosphorylation was observed in the following experimental conditions: (a) the ATP concentration was raised from 10 μM to 2 mM, (b) the reaction was carried out at 0 or 37°, (c) NaCl or KCl was added to the control medium up to a concentration of 120 mM. Thus, in the subsequent experiments, only the Ca^{2}-dependent phosphorylation will be reported, *i.e.*, the amount of E∼P formed in the presence of Mg^{2+} and Ca^{2+} subtracted from the amount of E∼P formed in the presence of only Mg^{2+} (Makinose, 1969).

ATP Dependency of the P_i Transfer Reaction. Table I shows data of Ca^{2+} uptake, Ca^{2+}-dependent ATPase activity, and Ca^{2+}-dependent phosphorylation of the membrane measured simultaneously in different microsome preparations using an optimal Ca^{2+} concentration. The Ca^{2+} uptake and ATPase activity proceeded at a fairly slow rate at 0°. At 37°, these two activities increased three- to fourfold when the ATP concentration was raised from 4 μM to 2 mM (de Meis, 1971; Weber *et al.*, 1966). However, no significant differences were found for the amount of P_i transfered to the microsomal membrane in the different experimental conditions described in the table. In several experiments, the ATP concentration was lowered to 2 μM. Provided that no monovalent cation was added to the medium, the amount of E∼P was the same as that shown in Table I. Thus, we failed to measure the ATP apparent K_m for reaction 1 shown above.

10 μM ATP. INHIBITION OF THE P_i TRANSFER REACTION BY Na^+ AND K^+. Figure 1 (left) shows that using an ATP concentration of 10 μM, at 0°, Na^+, K^+, and Li^+ inhibit the Ca^{2+}-dependent phosphorylation of the membrane. Essentially the same degree of inhibition was observed when either sodium chloride or sodium acetate were used. No inhibition was observed when 40 mM choline chloride was added to the control medium. Thus, the inhibition observed was specifically related to the monovalent cation used, and was not related to the osmotic balance of the system. In a previous report (de Meis, 1971) it has been shown that the alkali ions inhibit the Ca^{2+}-dependent ATPase activity of the vesicles in the order $Na^+ > K^+ \geq Li^+$. Figure 1 shows a similar sequence of inhibitory activity for the phosphorylation of the membrane.

Figure 1 (right) shows a similar experiment performed at 37°. The maintenance of the level of E∼P over 1-min incubation after the addition of Ca^{2+} shows that the regenerative system used assured the maintenance of the ATP concentration in the assay medium. Similarly to the experiment at 0°, Na^+ and K^+ inhibited the phosphorylation of the membrane. However, when compared with the data at 0°, the inhibitory activity of Na^+ was only slightly higher than that of K^+. This can also be seen in Figures 2 and 3. It has been shown that Cl^- inhibits the ATP: creatine phosphotransferase activity (Noda *et al.*, 1960; Heyde and Morrison, 1970). Thus, in these experiments, sodium or potassium acetate was used.

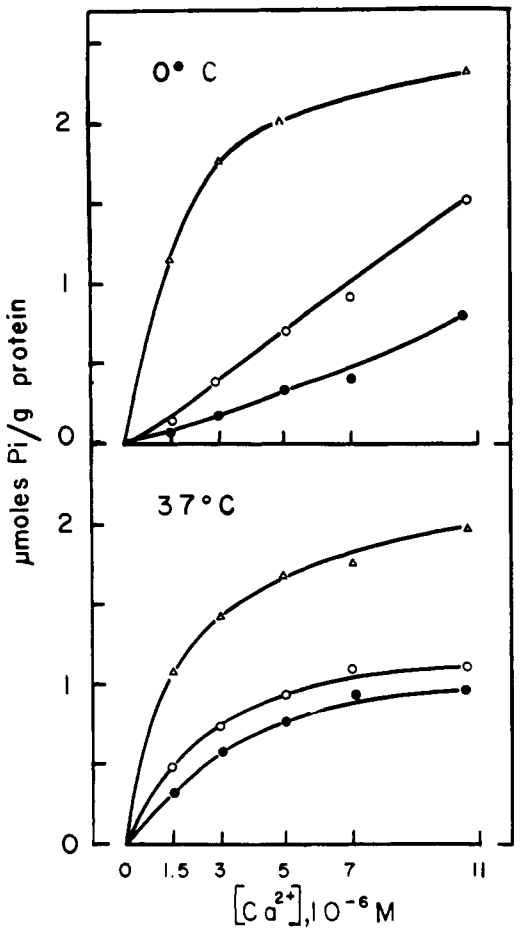

FIGURE 2: Ca^{2+} dependence of E~P formation and its inhibition by Na^+ and K^+ at ATP 10 μM. For zero Ca^{2+}, no $CaCl_2$ was added and the EGTA concentration was 0.70 mM. For the calculated free Ca^{2+} concentration of 1.5, 3.0, 5.0, 7.0, and 10.6 μM; 0.2 mM $CaCl_2$, and 0.70, 0.46, 0.35, 0.30, and 0.26 mM EGTA, respectively, were added to the assay medium. Other additions and experimental conditions were as described under Methods. Upper: The reaction was carried out at 0°. —△— Control medium; —○— control medium plus 40 mM KCl; —●— control medium plus 40 mM NaCl. Below: The reaction was carried out at 37°. —△— Control medium; —○— control medium plus 40 mM potassium acetate; —●— control medium plus 40 mM sodium acetate.

Figure 2 shows that the formation of E~P in the control media increases as a function of Ca^{2+} concentration (Makinose, 1969). The Ca^{2+} concentration in which the phosphorylation reaches its half-maximal value varied between 1.5 and 2.0 μM both at 0 and 37°. Maximal phosphorylation was obtained by using a free Ca^{2+} concentration of 10.6 μM. Addition of an excess of Ca^{2+} (up to 200 μM) to the assay medium did not alter the amount of E~P formed.

Figures 2 and 3 show that the inhibitory activity of Na^+ and K^+ decreases progressively when the free Ca^{2+} concentration of the assay medium is raised. For the lower Ca^{2+} concentrations, the inhibitory activity of Na^+ and K^+ was higher at 0° than at 37°. This was seen particularly with the use of Na^+. Thus, differently shaped saturation curves for Ca^{2+} dependence were observed at 0 and 37° when these ions were added to the control medium.

Figure 3 shows that for 1.5 μM Ca^{2+}, 50% inhibition was observed at 0° upon addition of 10 mM Na^+ or 20 mM K^+. The inhibitory activity of these cations decreased when the Ca^{2+} concentration was raised to 10.6 μM and in the presence

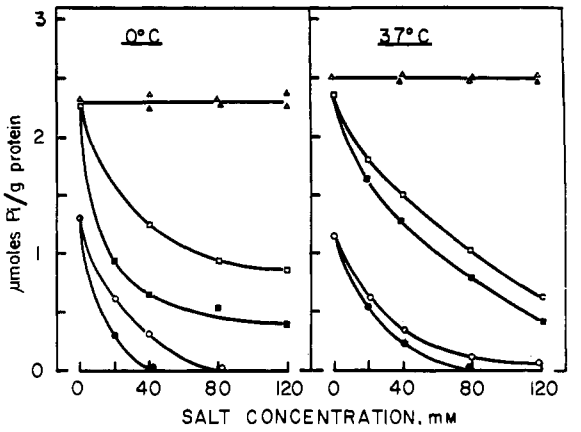

FIGURE 3: Correlation between the Ca^{2+} concentration and the inhibitory activity of Na^+ and K^+ at ATP 10 μM. The assay medium composition and the experimental conditions were as described under Methods. —○— 0.7 mM EGTA plus 0.2 mM $CaCl_2$; the calculated free Ca^{2+} concentration was 1.5 μM. —□— 0.26 mM EGTA plus 0.20 mM $CaCl_2$; the calculated free Ca^{2+} concentration was 10.6 μM. —△— The $CaCl_2$ concentration was 0.10 mM. No EGTA was added. Open symbols, KCl (right) or potassium acetate (left). Closed symbols, NaCl (left) or sodium acetate (right). The reaction was performed at 0° (left) or at 37° (right).

of 100 μM Ca^{2+}, addition of up to 120 mM NaCl to the control medium did not modify the degree of phosphorylation of the membrane. Similar results were observed when the reaction was carried out at 37°. Ouabain, up to a concentration of 1 mM, neither altered the phosphorylation reaction when added to the control medium nor interfered with the inhibition promoted by Na^+ or K^+.

2 mM ATP. INHIBITION OF THE P_i TRANSFER BY Na^+ OR K^+. Figure 4 shows the phosphorylation of the membrane pro-

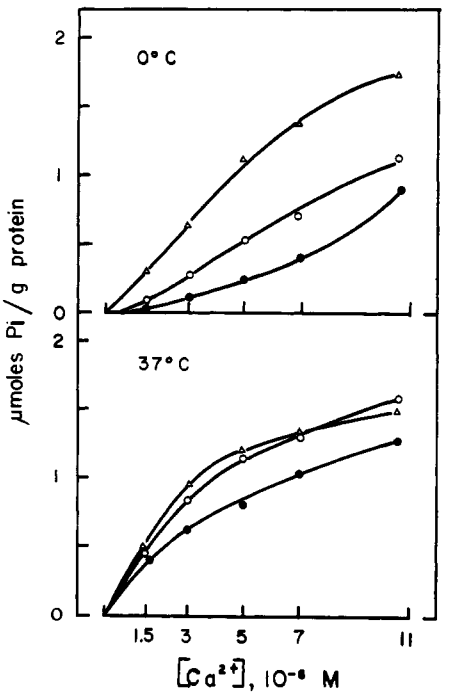

FIGURE 4: The Ca^{2+} dependence of E~P formation at ATP 2 mM. The $CaCl_2$ and EGTA concentrations were as described in Figure 2. Other additions and experimental conditions were as described under Methods. —△— Control medium; —○— control medium plus 40 mM KCl; —●— control medium plus 40 mM NaCl. The reaction was performed at 0° (upper) or at 37° (below).

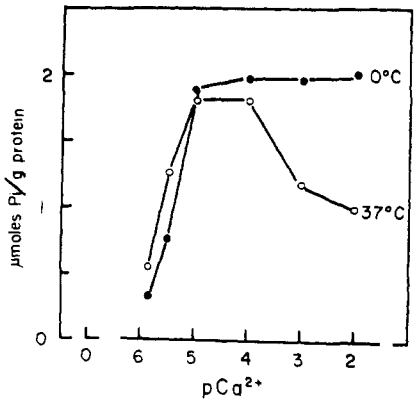

FIGURE 5: Temperature dependence on the inhibition by excess of Ca^{2+}. The ATP concentration was 2 mM. Other additions were as described for the control medium under Methods. Reaction performed at 0° (—●—) and at 37° (—○—).

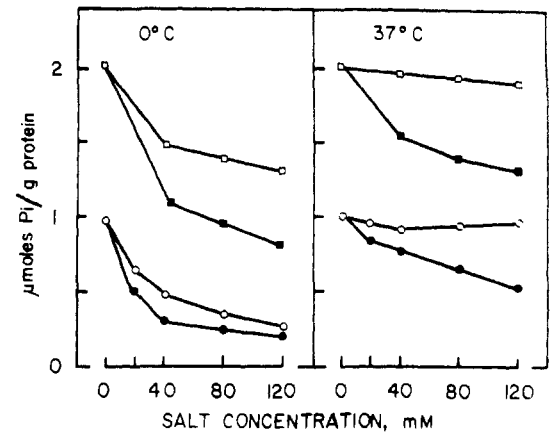

FIGURE 6: Correlation between Ca^{2+} concentration and the inhibitory activity of Na^+ and K^+ at 2 mM ATP. Additions and experimental condition were as described under Methods. Open symbols, KCl. Closed symbols, NaCl. Left: The reaction was performed at 0°. —○— 0.39 mM EGTA plus 0.20 mM $CaCl_2$, the calculated free Ca^{2+} concentration was 4 µM: —□— 0.26 mM EGTA plus 0.20 mM $CaCl_2$, the calculated free Ca^{2+} concentration was 10.6 µM. Right: The reaction was performed at 37°. —○— 0.70 mM EGTA plus 0.20 mM $CaCl_2$, the calculated free Ca^{2+} concentration was 1.5 µM; —□— 0.26 mM EGTA plus 0.20 mM $CaCl_2$. The calculated free Ca^{2+} concentration was 10.6 µM.

tein as a function of Ca^{2+} concentration. At 37°, the shape of the curve resembled that obtained using low ATP concentration (Figure 2). Half-maximal phosphorylation was reached with Ca^{2+} concentration of 1.5–2.0 µM. At 0°, the slope of the curve was lower and half-maximal phosphorylation was observed using Ca^{2+} concentrations in the range 3.5–4.5 µM.

Figure 5 shows that for both temperatures, maximal phosphorylation was obtained by using a free Ca^{2+} concentration of 10 µM. Addition of excess Ca^{2+} resulted in inhibition of the phosphorylation. However, this was observed only when the reaction was performed at 37°.

Figures 4 and 6 show that the Na^+ or K^+ inhibition of the ^{32}P transfer reaction in high ATP at 0° was similar to that observed in low ATP media at any temperature. A comparison with Figures 2 and 3 will clarify this point. However, when the temperature was raised to 37°, no K^+ inhibition could be detected in the high ATP media, and Na^+ inhibition became much attenuated.

LiCl, up to 120 mM, produced little or no inhibition of the membrane phosphorylation both at 0 and 37°.

Discussion

Inhibition by Excess Ca^{2+}. Figure 5 shows that excess of Ca^{2+} inhibits reaction 1 only when performed at 37°. This inhibition does not seem related to a competition between the ions Mg^{2+} and Ca^{2+} (Martonosi, 1969; Inesi *et al.*, 1970; Pucell and Martonosi, 1971). It is difficult to explain why such a competition was observed only at 37°. It has been shown that when a high Ca^{2+} concentration is reached inside the sarcoplasmic vesicles, the Ca^{2+}-dependent ATPase is inhibited (Makinose and Hasselbach, 1965; Weber *et al.*, 1966). It is possible that a similar phenomenom accounts for the data of Figure 5. At 0°, the Ca^{2+} transport is very small. Therefore it is unlikely that a large amount of Ca^{2+} accumulated inside the vesicles over the 10-sec incubation.

Ca–EGTA Dissociation Constant. In order to study the phosphate transfer reaction as a function of Ca^{2+}, a $CaCl_2$–EGTA buffer was used. This was required in order to minimize the variation of free Ca^{2+} concentration in the assay medium due to Ca^{2+} absorption and Ca^{2+} transport by the vesicles (Hasselbach, 1964).

Different values for the dissociation constant (K_d) have been reported (Schwarzenbach *et al.*, 1957; Holloway and Reilley, 1960; Ebashi, 1961; Ogawa, 1968). We used the value reported by Ebashi (1961) and Ogawa (1968). The free Ca^{2+} concentration for half-maximal phosphorylation was found in our experimental conditions to be 1.5–2.0 $\times$ 10^{-6} M. Makinose (1969) and Inesi *et al.* (1970) reported half-maximal phosphorylation with a free Ca^{2+} concentration of 1 $\times$ 10^{-7} M. However, these authors used the K_d of 2 $\times$ 10^{-7} and 3.2 $\times$ 10^{-7} M, respectively, for their calculations. These constants are higher than the one used in this study. Thus, our data agree substantially with those of Makinose (1969) and Inesi *et al.* (1970). The same range of free Ca^{2+} concentration for half-maximal phosphorylation would be obtained if our values of free Ca^{2+} for a given mixture of $CaCl_2$ and EGTA were calculated with the K_d used by these authors.

In a previous paper (de Meis and Hasselbach, 1971) it was shown that Na^+, K^+, and Li^+ do not interfere with the K_d of Ca–EGTA. Murphy and Hasselbach (1968) studied the effect of temperature on this constant.

Inhibition by Na^+ and K^+. The data presented show that Na^+ and K^+ inhibit reaction 1. The degree of inhibition varied with the temperature in which the assay was performed and with the ATP and Ca^{2+} concentrations. On the basis of these data, the following working hypothesis is proposed. (a) The binding of large amounts of ATP to the membrane promotes a conformational change of the Ca^{2+} transport system. This was only observed at 37°. (b) The conformational change modifies the specificity of the Ca^{2+} binding site. Before the change (0°, ATP 10 µM or 2 mM, and 37°, ATP 10 µM), the different ions would be able to bind to the active site, though only Ca^{2+} activated the P_i transfer reaction. Depending on the amount of Na^+ and K^+ in the medium, the reaction was inhibited due to competition of these ions with Ca^{2+} for the site. When the conformational change takes place (37°, ATP 2 mM) K^+ would no longer be able to bind to the site and Na^+ would bind poorly. According to the Michaelis–Menten kinetics (rigid site) one should expect that using a free Ca^{2+} concentration in order to obtain half-maximal phosphoryla-

tion (apparent K_m), Na^+ and K^+ would always compete with Ca^{2+} for the binding site in any of the experimental conditions tested. The same applies if ATP modifies the Ca^{2+} affinity of the enzyme (Yamamoto and Tonomura, 1967; Inesi *et al.*, 1970). In the literature there is additional evidence that the Ca^{2+} transport system exhibits characteristics similar to those described for allosteric enzymes. Yamamoto and Tonomura (1967), studying the kinetics properties of the Ca^{2+}-dependent ATPase activity of the sarcoplasmic reticulum, have concluded that at high concentrations ATP acts upon the enzyme not only as substrate but also as a regulator which controls the binding of substrate and the rate of decomposition of the phosphorylated enzyme. Inesi *et al.* (1967, 1970), measuring the Ca^{2+} transport and Ca^{2+}-dependent ATPase and phosphoprotein formation as a function of the ATP concentration, found a two-step curve suggesting a substrate activation of the Ca^{2+} transport system. These authors included KCl in their assay medium. In this and previous papers, it was shown that at low ATP concentration, K^+ inhibits the three parameters. It is possible that the apparent low affinity for Ca^{2+} in presence of low ATP concentrations (Inesi *et al.*, 1970) is related to a competition between K^+ and Ca^{2+}. Weber (1968) by means of the heavy fraction of sarcoplasmic reticulum, has shown that the effect of caffeine on Ca^{2+} transport varies with the ATP concentration in the assay medium.

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