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MECHANISM OF ACTIVE SHRINKAGE IN MITOCHONDRIA
II. COUPLING BETWEEN STRONG ELECTROLYTE FLUXES

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SUMMARY

1. Addition of succinate to valinomycin-treated mitochondria incubated in KCl causes a large electrolyte penetration. The process depends on a steady supply of energy and involves a continuous net extrusion of protons. Rates of respiration and of electrolyte penetration proceed in a parallel manner.

2. A passive penetration of K^+ salt of permeant anions occurs in respiratory-inhibited mitochondria after addition of valinomycin. Addition of succinate at the end of the passive swelling starts an active extrusion of anions and cations with restoration of the initial volume. The shrinkage is accompanied by a slow reuptake of protons. The initiation of the active shrinkage correlates with the degree of stretching of the inner membrane. The extrusion of electrolytes is inhibited by nigericin, while it is only slightly sensitive to variations of the valinomycin concentration larger than two orders of magnitude.

3. Passive swelling and active shrinkage occurs also when K^+ is replaced by a large variety of organic cations. The rate of organic cation penetration is enhanced by tetraphenylboron, while the rate of electrolyte extrusion is insensitive to variation of the tetraphenylboron concentration.

4. Active shrinkage, either with K^+ or organic cation salts, is inhibited by weak acids. The phosphate inhibition is removed by SH inhibitors. The active shrinkage is also inhibited by mersalyl to an extent of about 60 %.

5. Three models of active shrinkage are discussed: (a) mechanoprotein, (b) electrogenic proton pump, and (c) proton-driven cation anion pump.

INTRODUCTION

Azzi and Azzone [1] reported a passive swelling in valinomycin–alkaline–treated mitochondria incubated in KCl and an active shrinkage after addition of succinate. Brierley [2] observed that supply of energy caused a marked enhancement of swelling and suggested that anion permeability was increased through an energy

linked alkalinization, either in the matrix or in the membrane. Brierley then proposed that: (a) the KCl influx was a passive process and (b) KCl efflux was due to operation of the electrogenic proton pump through an electrophoretic diffusion of Cl^- and an H^+/K^+ exchange. We shall now show that: (a) the electrolyte influx is strictly coupled to energy supply; (b) the electrolyte efflux is inhibited by either nigericin or weak acids and (c) K^+ can be replaced by a number of organic cations which are assumed to undergo electrophoretic transport. It is possible to explain these phenomena on the basis of two assumptions: first that the inner membrane possesses a proton-driven, cation-anion, pump (cf. ref. 3); second that a passive H^+ influx, through a H^+ ion permeable membrane, is electrically coupled with the uptake of strong acids and the extrusion of strong bases.

EXPERIMENTAL

Material and methods were similar to those used in the preceding paper [3]. Liver mitochondria were prepared in a sucrose/EDTA/Tris medium, washed twice and resuspended free of EDTA. Penetration into and extrusion of solutes from mitochondria was followed continuously by recording the absorbance change at 600 nm in a Hitachi Perkin Elmer spectrophotometer model 124. The correspondences between photometric and volume changes were always controlled by gravimetric measurements. For this purpose the volume of the matrix space was determined by correcting the weight of the mitochondrial pellet for the amount of mitochondrial protein and the volume of [^{14}C]Dextran, M_r 60 000. The absorbance changes were converted into solute fluxes rates either by the procedure of Klingenberg et al. [5] or by that of Massari et al. [6, 7].

The penetration and extrusion of cations was determined with ^{86}Rb. Movement of H^+ was recorded continuously by two methods: (a) with the H^+ electrode; (b) with the acid-base indicator bromcresol purple at a concentration of 10 μM (measuring wavelengths were 588 and 622 nm). In case (a), a Radiometer pH meter Model 26 was used. In case (b) a dual wavelength spectrophotometer was used. Oxygen uptake was measured with a Clark oxygen electrode in thermally equilibrated cuvettes. The organic cations were obtained commercially mainly from Fluka and Eastman Kodak.

RESULTS

In Fig. 1 is depicted schematically the model system analyzed in the present paper. Respiratory inhibited mitochondria were incubated in a medium containing 30 mM K salts of univalent inorganic anions (Cl^-, NO_3^-, Br^-, I^-, SCN^-). Addition of valinomycin caused a large swelling if the anion was permeant or the pH was alkaline [2] (Fig. 1B). The rate of anion permeation increased with the Hofmeister series. Only a partial swelling was caused at pH 7.5 in the presence of Cl^-. However, in accord with Brierley [3], a rapid swelling was initiated by the addition of succinate (Fig. 1A). Under the conditions of Fig. 1B, addition of succinate at the end of the swelling phase initiated a shrinkage. Under the conditions of Fig. 1A, after a phase of swelling, the mitochondria spontaneously reversed to a phase of shrinkage. Thus in both cases under aerobic conditions the initial osmotic volume was restored.

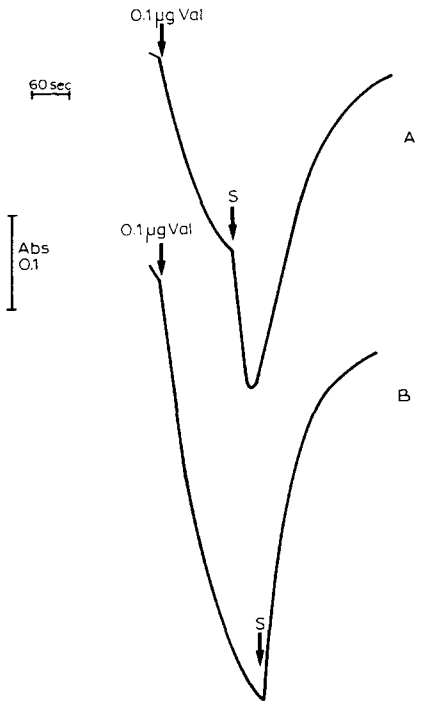

Fig. 1. Schematic representation of swelling-shrinkage cycles in presence of K^+ salts of univalent inorganic anions. The medium contained in A 30 mM KCl, 10 mM Tris/Cl, 1 mM EDTA pH 7.5, 1 μM rotenone and 1.6 mg mitochondrial protein. Swelling was initiated by 0.1 μg valinomycin. 1 mM succinate was then added. In B, KCl was replaced with KI and 2 mM succinate was added at the end of the swelling phase. Final volume, 2 ml. Val and S indicate valinomycin and succinate, respectively.

The swelling phase

Brierley observed a rapid burst of H^+ ion extrusion followed by a reuptake after addition of valinomycin to aerobic mitochondria in KCl at neutral pH. In this system there is overlapping of two phenomena, the swelling and the shrinkage phases. In order to make a proper correlation between H^+ ion extrusion and electrolyte movement both parameters were measured at pH 6.5 where the shrinkage phase is abolished. Fig. 2A shows that swelling was accompanied by H^+ ion extrusion. Fig. 2B shows the effect of valinomycin on the extent of H^+ ion extrusion and Cl^- penetration. It is seen that the amount of H^+ extrusion was of the order of 20–25 μmol · g protein^{-1} and was only slightly affected by the valinomycin concentration. On the other hand, the extent of Cl^- penetration was markedly dependent on the valinomycin concentration and amounted to about 4 times the H^+ extrusion at high valinomycin concentrations.

H^+ extrusion as well as KCl uptake required a continuous supply of energy. Addition of carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) or of antimycin A abolished the H^+ ion extrusion and caused a partial reuptake of the H^+ extruded, say of the order of 60 %. A reuptake of H^+ was also induced by nigericin.

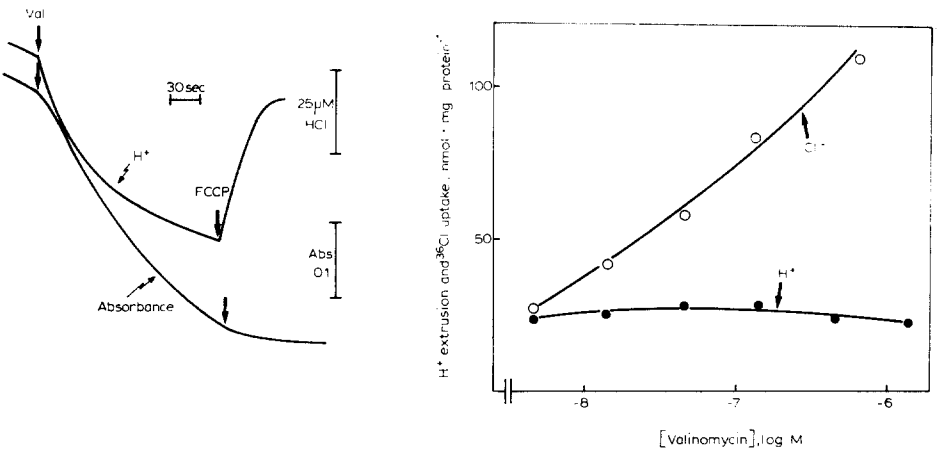

Fig. 2. Correlation between H^+ extrusion and KCl uptake. The medium contained 50 mM KCl, 2 mM Tris/Cl, 0.5 mM EDTA pH 6.5, 1 μM rotenone. In the left part were added 2 mM succinate, and then 0.001 μg valinomycin. In the right part were added 2 mM succinate and variable amounts of valinomycin. Amount of protein was 2 mg in the absorbance experiment and 3 mg in the H^+ expeperiment. The H^+ ion movements were measured with Bromcresol purple. The uptake of KCl was calculated as described in the Methods. In the left part the H^+ ion was measured with the electrode and 4.9 mg protein were used for absorbance and H^+ ion measurement.

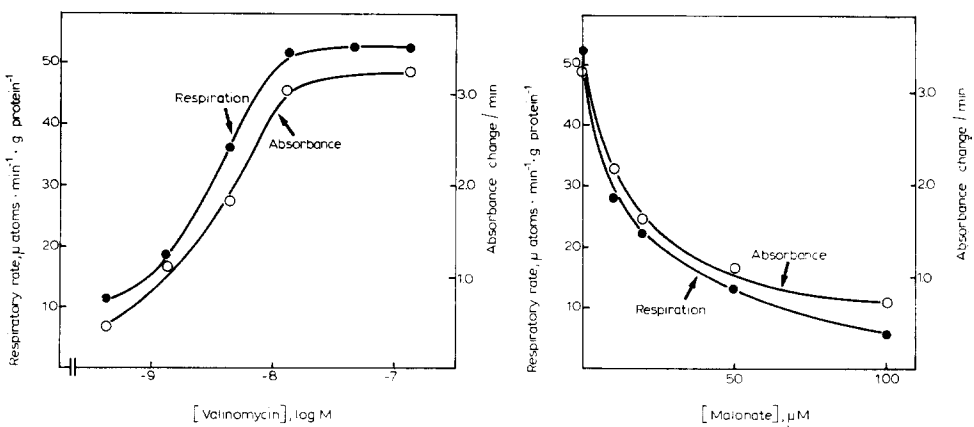

Fig. 3A and B. Relation between rates of respiration and of KCl uptake in presence of various valinomycin and malonate concentrations. Experimental conditions as in Fig. 2 except that Tris was 10 mM. The reaction was always initiated with 2 mM succinate. Valinomycin and malonate were added before succinate. Amount of protein was 2.0 mg during measurements of absorbance changes and 4 mg during measurements of oxygen uptake. Final volume 2 ml.

Interruption of energy supply and reuptake of H^+ were accompanied by inhibition of the electrolyte uptake at any stage of the swelling process.

Figs 3A and B show a correlation between rates of KCl penetration and respiration. In Fig. 3A the rate of KCl penetration increased proportionally to the valinomycin concentration and the same happened for the respiratory rate. The rate of KCl penetration amounted to about 500 μmol · min^{-1} · g $protein^{-1}$ at the

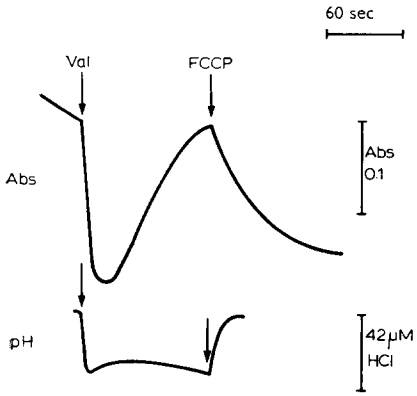

Fig. 4. Kinetics of H^+ ion movement during active shrinkage. The medium contained 30 mM KNO_3, 1 mM Tris/Cl, 0.5 mM EDTA pH 7.5, 1 μM rotenone, 1 mM succinate. The swelling was initiated by 0.1 μg valinomycin when indicated 1 μM FCCP. Absorbance was recorded in an Eppendorf photometer at 546 nm. pH was recorded with the pH electrode. Final volume, 2ml. 3,3 mg protein.

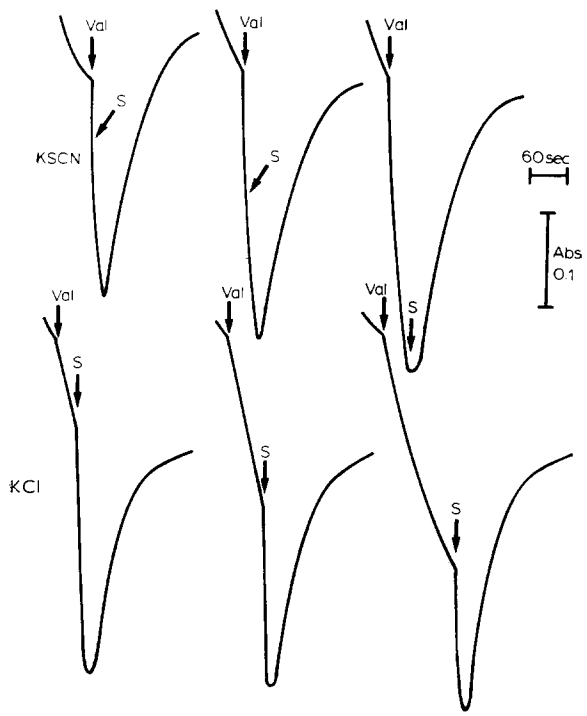

Fig. 5. Dependence of initiation of shrinkage on extent of membrane stretching. In the upper part the medium contained 30 mM KSCN, 10 mMTris/Cl, 1 mM EDTA, pH 7.5, 1 μM rotenone. Swelling was initiated with 0.01 μg valinomycin while 1 mM succinate was added after 5, 15 and 50 s., respectively. In the lower part KSCN was replaced with KCl and 1 mM succinate was added after 30, 60 and 120 s, respectively. Final volume 2 ml, 1.8 mg protein.

TABLE I

ACTIVE EXTRUSION OF Rb^+

The medium contained 30 mM $RbNO_3$, 10 mM Tris/Cl pH 7.5, 1 mM EDTA, 2 μM rotenone, variable amounts of valinomycin and 12.9 mg mitochondrial protein. Final volume, 2.0 ml. In the passive swelling series the samples were left for 180 s and then centrifuged. In the active shrinkage series the samples, after completion of swelling, were bubbled with oxygen, supplemented with 4 mM succinate and 1 mM ATP, and centrifuged after 90 s. The water content was measured gravimetrically and the values corrected for the Dextran-permeable space determined with [^{14}C]Dextran. Rb^+ was measured with $^{86}Rb^+$.

Additions	$H_2O \cdot \mu l \cdot mg\ protein^{-1}$			$Rb^+ \cdot nmol \cdot mg\ protein^{-1}$		
	−succ · ATP	+succ · ATP	Δ	−succ · ATP	+succ · ATP	Δ
0.01 μM valinomycin	1.66	0.29	−1.37	84	55	−29
0.04 μM valinomycin	1.77	0.56	−1.21	92	57	−35
0.1 μM valinomycin	1.80	0.58	−1.22	93	62	−31
0.4 μM valinomycin	2.29	0.88	−1.41	103	69	−34

highest valinomycin concentrations. This rate may be compared with a maximal respiratory rate of about 60 μatoms oxygen · min^{-1} · g $protein^{-1}$ with a K^+/oxygen ratio of 8 with succinate as a substrate [8–9]. The experiment thus shows that the extent of stimulation of the respiration is proportional to the energy supply for KCl uptake. Fig. 3B shows that the inhibition of succinate oxidation caused by increasing malonate concentration caused a parallel decrease of the rate of KCl penetration. Thus in this case it was the limitation of the respiratory rate which reduced the uptake of electrolytes. Malonate caused a decrease of the extent of H^+ extrusion of about 40 %.

The shrinkage phase

Fig. 4 shows the correlation between H^+ and electrolyte movements at pH 7.5 where the swelling phase shifts to the shrinkage phase. It is seen that there was a rapid burst of H^+ extrusion, followed by a levelling off and then by a phase of slow H^+ reuptake. The reversing of direction of the H^+ movement preceeded the initiation of the shrinkage phase. At the end of the shrinkage FCCP caused on one side a reuptake of H^+ and on the other started again a swelling phase. The swelling rate, however, rapidly decreased. This is presumably due to a lowering of the anion permeability.

The rate of shrinkage was markedly dependent on the succinate concentration and on the pH of the medium. Below pH 7 the rate of shrinkage was negligible. The apparent K_m for succinate was 2 mM. In the preceeding paper it has been reported that the initiation of shrinkage depends on the extent of membrane stretching. In the experiment of Fig. 5 succinate was added after various period of times, either at the beginning or at the end of swelling phase, in the presence of KSCN or KCl. Only the

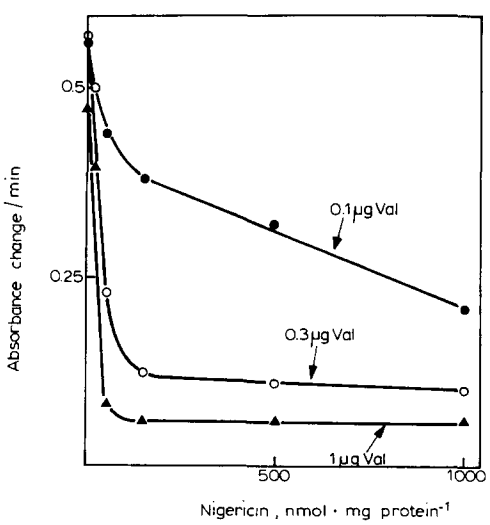

Fig. 6. Effect of nigericin on active shrinkage. The medium contained 30 mM KSCN, 10 mM Tris/Cl, 1 mM EDTA, pH 7.5, 1 μM rotenone. The passive swelling was initiated by an amount of valinomycin as indicated in the figure. After the attainment of equilibrium were added variable amounts of nigericin and then 1 mM succinate. Final volume, 2 ml, 1.6 mg protein.

more representative experiments are reported. In the presence of SCN^-, succinate added 5 s after valinomycin, i.e. before the occurrence of the passive swelling phase, was unable to start the shrinkage. On the other hand when added 50 s after valinomycin, i.e. after the end of swelling phase, succinate did start immediately the shrinkage. With KCl the shrinkage was always preceeded by a further swelling with a complementarity between the passive swelling before succinate and the active swelling after succinate. The less swollen was the mitochondria before succinate, the more extensive was the phase of active uptake. The shrinkage phase started in most cases at about the same degree of swelling. This presumably corresponds to the penetration of a certain amount of Cl^- and to the attainment of a minimal degree of membrane stretching.

Table I shows the changes of water and electrolyte content due to active shrinkage. As discussed in the preceeding paper [3] ATP was used to avoid the consequences of anaerobiosis in the suspension. However the extent of absorbance and volume changes was not affected by the presence of ATP. The amount of water and of Rb^+ in the matrix increased with the amount of valinomycin. Addition of succinate+ATP caused an extrusion of Rb^+ and of osmotically equivalent amounts of water. The extrusion was independent of the valinomycin concentration.

Fig. 6 shows that addition of nigericin caused a marked inhibition of the rate

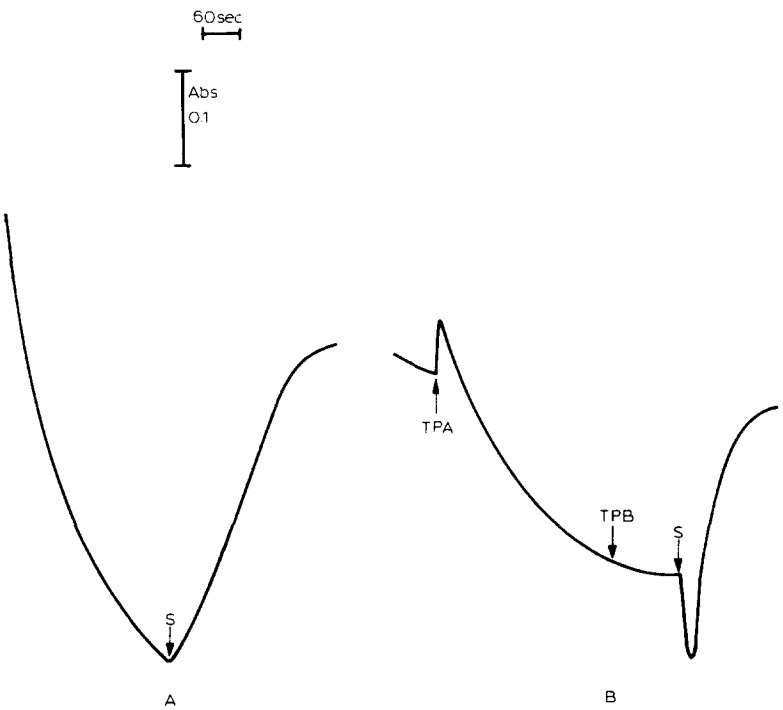

Fig. 7. Active shrinkage in presence of tetrapropylammonium. In A the medium contained 30 mM tetrapropylammonium NO_3^-, 10 mM Tris/Cl, 1 mM EDTA pH 7.5, 1 μM rotenone. Shrinkage was initiated with 1 mM succinate. In B the medium contained 30 mM KNO_3, 10 mM Tris/Cl, 1 mM EDTA, pH 7.5, 1 μM rotenone. Where indicated 15 mM tetrapropylammonium chloride, (TPA) 5 μM tetraphenylboron (TPB) and then 1 mM succinate were added. Final volume, 2 ml, 1.8 mg protein.

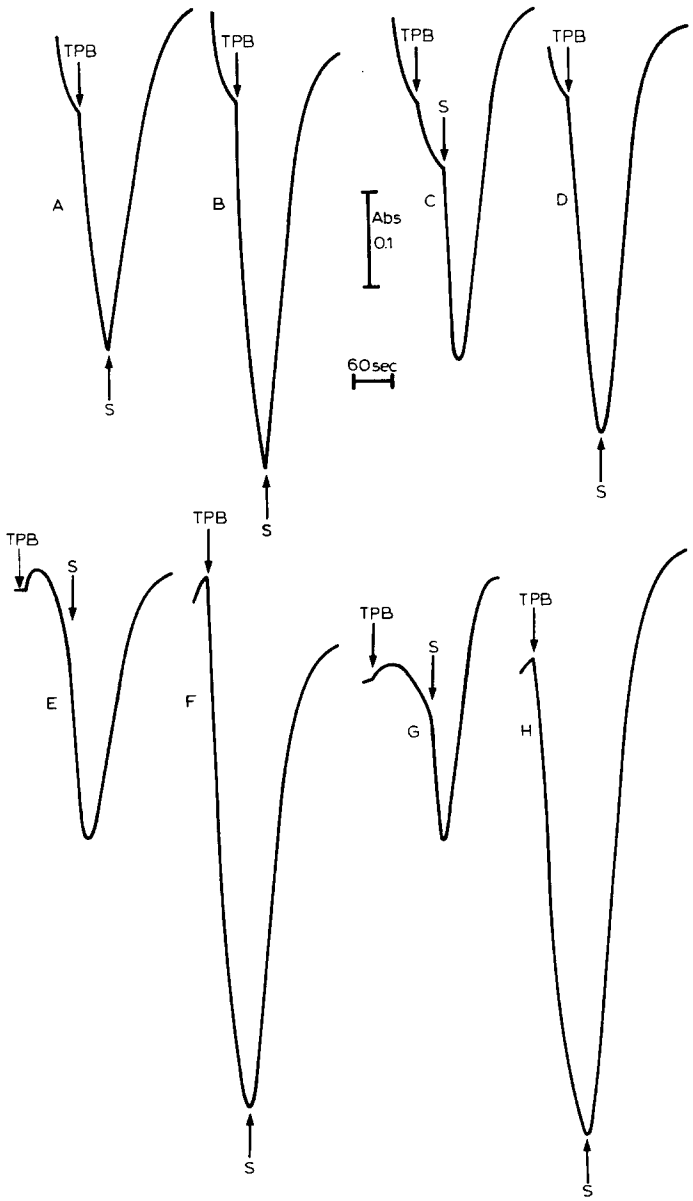

Fig. 8. Passive swelling and active shrinkage in presence of organic cations and tetraphenylboron. The medium contained in A and B 30 mM tetrapropylammonium NO_3^-, in C and D 30 mM tetrapropylammonium chloride, in E and F 30 mM benzyltriethylammonium chloride in G and H 30 mM trimethylphenylammonium chloride. In all experiments were present also 10 mM, Tris/Cl 1 mM EDTA, pH 7.5 and 1 μM rotenone. The amount of tetraphenylboron was 5 μM in A, C, E, G, and 20 μM in B, D, F, H. Where indicated was added 1 mM succinate. Final volume, 2 ml, 2 mg protein.

of active shrinkage. At high valinomycin concentration a very small amount of nigericin abolished almost completely the shrinkage, both in rate and in extent (not shown).

Fig. 7 shows that swelling and shrinkage cycles occur also in the presence of organic cations [10–12]. There was a spontaneous swelling of mitochondria incubated in NO_3^- salts of tetrapropylammonium, Fig. 7A, and shrinkage after addition of succinate. In Fig. 7B it is seen that swelling was initiated after addition of tetrapropylammonium to mitochondria incubated in KNO_3. Subsequent addition of succinate caused a further swelling followed by a shrinkage phase. In the upper part of Fig. 8 the swelling was obtained with the Cl^- and NO_3^- salts of tetrapropylammonium. In the lower part were used benzyltriethylammonium and trimethylphenylammonium. The swelling rate was markedly dependent on the nature of the anion and on the concentration of tetraphenylboron. With Cl^- as anion and at low tetraphenylboron concentrations the swelling was markedly accelerated by respiration as in the case of K^+/valinomycin. The extent of electrolyte penetration was a function of the tetraphenylboron concentration. However, either at low or at high tetraphenylboron concentration there was a complete restoration of the initial osmotic volume after addition of succinate. The matrix water, measured gravimetrically, in 12.5 mM, 25 mM, 37.5 mM and 50 mM tetrapropylammonium nitrate (plus 10 μM tetraphenylboron), was 3.2, 2.9, 1.6 and 1.2 $\mu l \cdot$ mg protein^{-1} after swelling, respectively. After shrinkage the matrix water was reduced to 1.5, 0.8, 0.4 and 0.2 $\mu l \cdot$ mg protein^{-1}, respectively.

In Fig. 9 the permeability to cation was varied within more than two orders of magnitude by changing the valinomycin concentration. The increase of valinomycin concentration affected only slightly the rate of shrinkage independently on the nature of anions. Particularly in the presence of Cl^- the effect of valinomycin was negligible. The rate of active shrinkage was also not affected when the concentration of tetra-

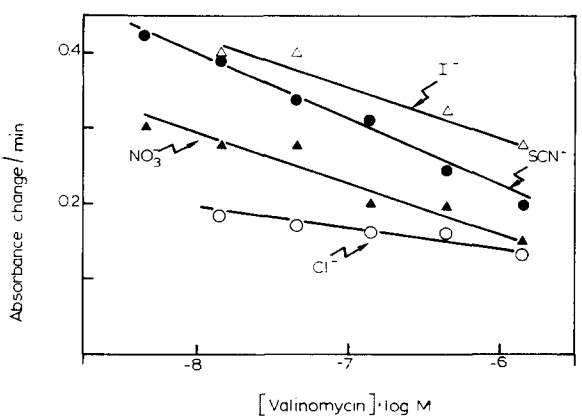

Fig. 9. Effect of various valinomycin concentrations on active shrinkage. The medium contained 30 mM of the K^+ salts of SCN^-, I^-, NO_3^-, and Cl^-, respectively, 10 mM Tris/Cl, 1 mM EDTA, pH 7.5 and 1 μM rotenone. Swelling was initiated by the concentrations of valinomycin indicated in the figure and active shrinkage by 1 mM succinate, 1.8 mg protein. In all cases active shrinkage was initiated at equal degrees of membrane stretching.

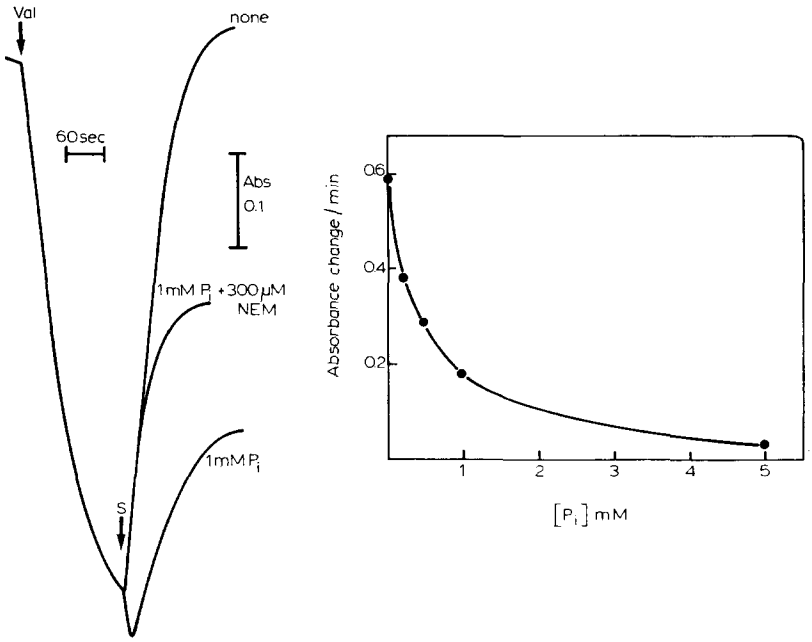

Fig. 10. Effect of P_i on active shrinkage. In the left part the medium contained 30 mM KI, 10 mM Tris/Cl,1 mM EDTA, pH 7.5, 1 μM rotenone and when indicated 1 mM P_i and 300 μM *N*-ethylmaleimide. Swelling was initiated with 0.03 μg valinomycin and active shrinkage with 1 mM succinate. In the right part were the same conditions except that the concentration of P_i was varied and *N*-ethylmaleimide was absent. Final volume 2 ml, 1.8 mg protein.

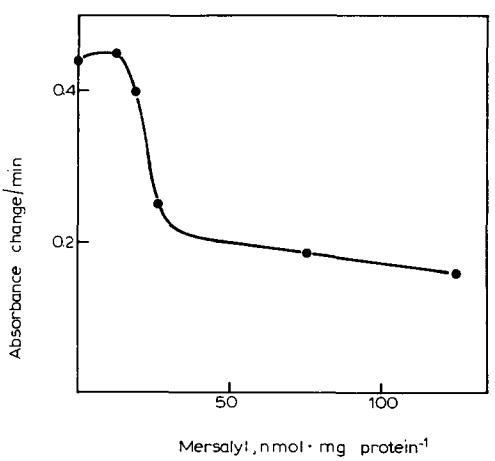

Fig. 11. Inhibition of shrinkage by mersalyl. The medium contained 30 mM KSCN, 10 mM Tris/Cl, 1 mM EDTA, pH 7.5 1 μM rotenone. Swelling was initiated with 0.1 μg valinomycin. At the end of the swelling the SH groups were titrated with the indicated amounts of mersalyl and active shrinkage initiated with 1 mM ascorbate plus 100 μM TMPD, 2 mg protein, final volume 2 ml.

phenylboron was varied in the presence of tetrapropylammonium within 2 and 20 μM whether the anion was Cl^- or NO_3 (not shown).

Brierley and Stoner [13] reported that anaerobic mitochondria did not swell in NH_4Cl at pH 8.3 but did swell in the presence of uncouplers. Furthermore, initiation of respiration caused shrinkage. We have carried out a similar experiment. Mitochondria were incubated in 30 mM KNO_3 and swelling initiated by 3 μg nigericin. The rate of swelling was low, presumably limited by the rate of H^+ permeation. Addition of succinate initiated a shrinkage. Addition of 0.03 μM FCCP, doubled the rate of swelling and inhibited about 40 % the rate of shrinkage.

Fig. 10 shows that the active shrinkage was very sensitive to the addition of inorganic phosphate. 1.0 mM P_i gave more than 50 % inhibition of the rate of active shrinkage in 30 mM KI. It is also seen in Fig. 10 that addition of 300 μM *N*-ethylmaleimide led to a partial restoration of the shrinkage extent. Acetate caused inhibition of shrinkage at low, and restoration of swelling at high concentrations [14].

Fig. 11 shows that the active extrusion of K^+ salt of univalent inorganic anions was inhibited by mersalyl. There was no inhibition below 20 nmol/mg protein, while an inhibition of about 60 % appeared between 20 and 30 nmol/mg protein. These concentrations are similar to those required to inhibit the active extrusion of P_i [3]. It was also tested whether the passive efflux of electrolytes induced by impermeant high molecular weight solutes was sensitive to mersalyl. However, at variance from the case of the P_i efflux no effect of mersalyl was observed.

DISCUSSION

The mechanoenzyme model

The mechanoenzyme model [15] does explain the extrusion of strong electrolytes. The model requires that, above a certain degree of stretching, the mechanoprotein starts operating and develops a hydrostatic pressure which compensates for the active uptake of cations. In the presence of nigericin or of weak acids, the operation of the mechanoenzyme is abolished by uncoupling or masked by cation uptake, respectively.

The electrogenic proton pump

The active swelling. As proposed by Brierley [3] respiration in valinomycin-treated mitochondria, in the absence of weak acid, leads to membrane damage and high rate of anion permeation.

On the other hand, the view that KCl penetration is a passive process is questionable. First, the electrogenic proton pump predicts that permeant strong acids are rather extruded by the membrane potential of the energized system, Fig. 12. Second, there is a strict correlation between respiration and anion uptake. The Cl^- penetration requires a steady supply of energy, while the rate of Cl^- penetration is a function of the respiratory rate. Nichols [16] has reported that nigericin causes KCl penetration in anerobic brown adipose tissue mitochondria due to the fatty acid-induced permeability to H^+ and Cl^-. Our experimental system recalls Nichols' system with two variations. First, the high rate of H^+ and Cl^- permeation is induced by respiration and not by fatty acids. Second, the H^+/K^+ exchange is not a passive

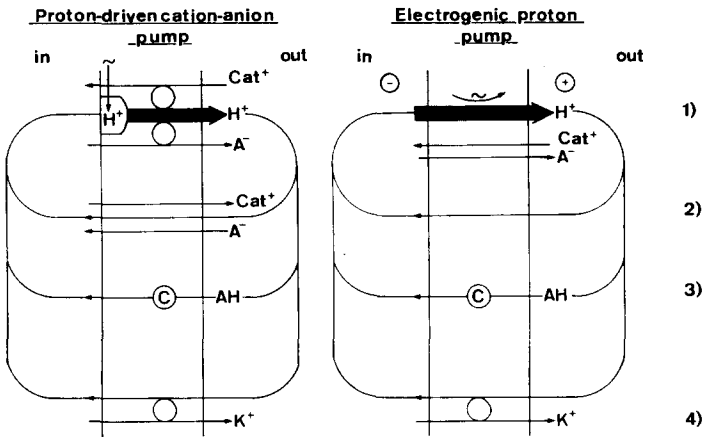

Fig. 12. Electrogenic proton pump and proton-driven cation-anion pump. (1) The electrogenic proton pump assumes an electrical coupling between primary proton movement and diffusion of permeable cations and anions. The proton-driven cation-anion pump assumes a direct coupling between proton movement and cations and anion movement. (2) The electrogenic proton pump assumes that passive H^+ permeation leads to decrease of $\Delta\tilde{\mu}_H$. The proton-driven cation-anion pump assumes that passive H^+ permeation leads under certain conditions to uptake of strong acids and extrusion of strong bases. (3) Indicates in both models the anion transport via carriers. (4) Indicates in both models the cation transport via natural antiporters or nigericin.

process catalyzed by nigericin, but an active process and provides the driving force for the HCl uptake.

The active shrinkage. The electrogenic proton pump model of Brierley [2, 13, 17] and Nichols [16] for the active shrinkage is that of an extrusion of anions from the matrix down an electrical potential and of cations, via a natural antiporter or nigericin, driven by a ΔpH, Fig. 12. Nichols [16] obtained active shrinkage after addition of BSA, considered to decrease the H^+ permeability. Nichols (16) considered a strong support for the electrogenic proton pump the fact that shrinkage was possible only under conditions of an electrical anion flux and of an electroneutral H^+/K^+ exchange and pointed out that shrinkage was abolished when the cation and anion fluxes appeared either as both electroneutral or both electrical.

In the mechanism of Brierley and Nichols, the extrusion of electrolytes depends on the balance of the cation and anion passive permeation rates. However, large variations of the cation permeation due to valinomycin and tetraphenylboron affect only slightly the rate of electrolyte extrusion with either lipophilic or hydrophilic anions (Fig. 10). This is not incompatible with an electrophoretic anion extrusion but requires that the rate of anion permeation is always much higher than the rate of cation permeation.

The model of Brierley [2, 13, 17] and Nichols [16] requires also a rapid H^+/K^+ antiporter. However, Douglas and Cockrell [18] have recently reported a very low rate of natural H^+/K^+ exchange in liver mitochondria. We have measured a rate of natural H^+/K^+ exchange, during swelling in potassium acetate, of about 10 μmol $\cdot$ min^{-1} $\cdot$ g protein^{-1}. This is less than ten times the rate of active K^+ extrusion. Furthermore, in the absence of nigericin the rate of K^+ extrusion through electro-

neutral H^+/K^+ exchange is negligible [3]. Two other observations are not easily explained by the electrogenic proton pump. First, increase of the rate of H^+/K^+ exchange by nigericin causes inhibition, rather than enhancement, of extrusion. Second, extrusion is independent of the chemical structure of the cation. Such a property has been taken to indicate that transport does not involve enzymic, or carrier interactions, but rather is electrophoretic in nature [11].

The proton-driven, cation-anion pump

An alternative view for the energy, linked movement of strong electrolytes is schematically depicted in Fig. 12. As for the uptake of strong acids, the proton-driven cation-anion pump assumes: a) a direct coupling between H^+ and cation fluxes and b) a high permeability to H^+. The energy linked H^+/K^+ exchange leads to a Δ pH. The Δ pH drives the uptake of H^+ which in turn drives the uptake of anions. The fluxes of H^+ and of anions are assumed to be electrically coupled.

As for the extrusion of strong bases, the proton-driven, cation-anion pump, assumes: (a) a direct coupling between H^+ and anion fluxes (cf. also ref. 3), and (b) a high permeability for H^+. The energy linked H^+–A^- efflux is assumed to involve a microscopical electroneutral flux, as in the case of the H^+/K^+ exchange, which leads to the formation of a ΔpH. This in turn drives the efflux of cations in two ways: in the case of the extrusion of NH_4NO_3 or of KNO_3+nigericin, as an electroneutral exchange. In the case of K^++valinomycin or of organic cations, by means of an electrical potential generated by the passive influx of H^+. The role of the ΔpH during shrinkage is emphasized by the inhibition of shrinkage by weak acids. This may be interpreted as being due to a decrease of ΔpH and therefore abolition of the driving force for H^+ influx and cation extrusion. The question arises about the factors causing the shift from active uptake to active extrusion. The present evidence indicates that the internal pH and the degree of stretching of the membrane play a significant role. Presumably pH and stretching render the permeability to anions from the matrix side higher than to cations from the outer side. thus determining a coupling of the proton pump to anion extrusion rather than to cation uptake. However, the capacity for cation uptake is still maintained as indicated by the nigericin inhibition which is due to energy dissipation caused by a valinomycin-nigericin dependent ion cycling.

Massari and Azzone [4] reported that swollen mitochondria have an equivalent pore radius at about 10 Å and concluded that an active ion extrusion mechanism is unlikely in such a membrane. The model of Fig. 12 requires that the H^+ influx is preferentially coupled to cation efflux rather than to anion influx since the opposite would lead to cycling of HCl. Also the active extrusion of P_i requires that K^+ efflux via nigericin be faster than P_i influx, (see effects of nigericin and SH inhibitors) [3]. Thus the weakest part of the model resides in the knowledge of the factors which determine the coupling of the cation and anion fluxes either to the pump or to the H^+ influx.

Sites for anion transport

On the basis of the effects of the SH inhibitors the following pattern seems to emerge: (a) most SH inhibitors inhibit the influx of P_i but not that of strong acids; (b) mersalyl inhibits the active extrusion but not the passive efflux of strong acids; the inhibition occurs at concentrations identical to those for the active P_i efflux. This

complex pattern may be explained by assuming that anion transport involves at least two steps: (a) one whereby the anion overcomes the hydrophobic barrier; (b) another whereby the anion interacts with the active transport system. The former site is involved during the passive transport of P_i but not of the strong acids while the latter is involved in the active extrusion of all anions.

The present observations explain other mitochondrial processes such as the mitochondrial oscillations which may be due to an alternation between active cation uptake and anion extrusion when there is membrane stretching [19].

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