Journal of Bioenergetics and Biomembranes, Vol. 10, Nos. 3/4, 1978

Swelling and Contraction of Heart Mitochondria Suspended in Ammonium Phosphate

Clinton D. Stoner[1] and Howard D. Sirak

Heart Biochemistry Research Laboratory, Department of Surgery, Ohio State University, Columbus, Ohio 43210

Received January 5, 1978

Abstract

Bovine heart mitochondria which have been allowed to swell in isotonic NH_4^+ phosphate contract in response to initiation of oxidative phosphorylation. The contraction occurs optimally at pH 6.0 and appears from inhibition studies to result from P_i uptake being slower than removal of internal P_i via phosphorylation of external ADP. Similar results are obtained when K^+ + nigericin is substituted for NH_4^+. Mersalyl inhibition of P_i transport in respiring, nonphosphorylating mitochondria which have been allowed to swell in NH_4^+ phosphate reveals a contractile process having an alkaline pH optimum. This contraction resembles closely the contraction observed in salts of strong acids and presumably occurs by electrophoretic ejection of P_i anions driven by electrogenic H^+ ejection.

[1]Mailing address: Dr. Clinton D. Stoner, Ohio State University, 1645 Neil Avenue, Columbus, Ohio 43210.

0145-479X/78/0800-0075$05.00/0

Introduction

Nonenergized mitochondria undergo rapid and extensive swelling when suspended in isotonic NH_4^+ phosphate [1–6]. This passive swelling results from external $H_2PO_4^-$ entering the mitochondria via the P_i carrier either with H^+ or in exchange for internal OH^-, and from NH_4^+ penetrating, in effect, by dissociating H^+ externally, entering the mitochondria as NH_3, and associating with internal H^+ formed as a result of the $H_2PO_4^-$ influx [1–6]. Mitochondria also swell passively in Na^+ and K^+ phosphate, but at much lower rates, particularly in the case of K^+ phosphate [3–6]. In these cases the cations appear to enter by way of an H^+/Na^+, K^+ exchange carrier having a higher affinity for Na^+ than for K^+ [3–6]. Consistent with this, nigericin, which catalyzes H^+/K^+, Na^+ exchange across the inner membrane [7,8], renders Na^+ and K^+ phosphate essentially equal to NH_4^+ phosphate in regard to mitochondrial swelling [3].

In contrast to nonenergized mitochondria, energized mitochondria undergo rapid and extensive swelling in an extremely wide variety of phosphoric acid salts [4–6]. The energy-dependent swelling has been well explained in terms of the chemiosmotic hypothesis by assuming that ΔpH drives the uptake of P_i via the P_i carrier, and that $\Delta\Psi$ drives the uptake of the cations by electrophoresis [4–6].

The present report concerns primarily the effects of mitochondrial metabolic activity on the volume changes of heart mitochondria suspended in NH_4^+ phosphate. It is shown that initiation of oxidative phosphorylation in respiring mitochondria which have been allowed to undergo high-amplitude swelling in this medium results in reversal of the swelling, the reversal going nearly to completion when the P_i carrier has been inactivated with mersalyl. This contractile process has a low pH optimum which distinguishes it from an energy-dependent, phosphorylation-independent contractile process having a high pH optimum. The latter appears to correspond to the high-amplitude mitochondrial contraction in P_i media described recently by Azzone et al. [9] and resembles closely the contraction observed in monovalent cation salts of strong acids [10–14].

The low-pH, phosphorylation-dependent contraction appears to correspond to the energy-dependent, low-amplitude, ADP-induced contraction observed in mitochondria which have been allowed to undergo energy-dependent, low-amplitude, P_i-dependent swelling in sucrose [15–18]. This swelling–contraction was detected originally by Packer [15] as a small decrease–increase in mitochondrial optical density (OD) and has been shown by Hackenbrock [16] to be associated with an increase–decrease in

the volume of the matrix. Further studies [17, 18] have shown the swelling–contraction to be associated with an increase–decrease in the concentration of internal P_i, indicating it to be osmotically produced. Since the swelling in these previous studies occurred against the osmotic pressure of sucrose, it is not entirely clear whether the contraction was due to energy-dependent removal of accumulated P_i or to passive efflux of the P_i as a result of respiratory energy being diverted from P_i uptake to phosphorylation. The findings of the present study indicate that the contraction may have been due wholly to energy-dependent removal of internal P_i, and that it may have occurred as a result of P_i uptake being slower than removal of internal P_i via phosphorylation of external ADP. A preliminary account of this work has been presented [19].

Methods

Bovine heart mitochondria were isolated according to a slight modification of the Nagarse procedure previously described [20]. Homogenates were prepared in media containing 250 mM sucrose, 0.1 mM EDTA, 10 mM TrisCl (pH 7.8 at 0°C), and 0.2 mg of Nagarse proteinase per gram of tissue. The mitochondria were washed twice and suspended finally at concentrations of 70 to 90 mg protein/ml in media containing sucrose and TrisCl at the concentrations given above.

Respiratory activity, mitochondrial OD (800 nm), and pH were monitored simultaneously in rapidly stirred suspensions (8 ml) contained in a closed, thermostated (30°C) reaction chamber [21]. In all cases the concentration of mitochondrial protein in the incubation mixture was 0.25 mg/ml. Other conditions of incubation are given with the results of the individual experiments reported. The reported changes in mitochondrial OD are assumed to give accurate indication of changes in mitochondrial matrix volume in accordance with the results of previous studies [22] showing the relationship between mitochondrial OD, packed volume, and ultrastructure. That this assumption is valid was ascertained for all major OD changes by the packed volume method [22] and in a few cases by electron microscopy.

Sucrose was obtained from Mallinckrodt Chemical Works and mersalyl (Na^+ salt), ADP (Na^+ salt), Krebs cycle acids, cytochrome *c* (Type IV), and hexokinase (Type F-300) from Sigma Chemical Company. Nigericin, S-13 and aurovertin were obtained as gifts from R. L. Harned (Commercial Solvents Corporation, Terre Haute, Indiana), P. Hamm (Monsanto Com-

pany, St. Louis, Missouri), and C. Baldwin (Dow Chemical Company, Zionsville, Indiana), respectively.

Results

Figure 1 presents typical OD and respiratory responses of heart mitochondria to suspension and incubation at pH 6.2 in media containing 0.1 M NH_4^+ phosphate, oxidizable substrates, and cytochrome *c*. Added cytochrome *c* is essential for maximum respiratory activity under these conditions, because the endogenous cytochrome *c* rapidly distributes throughout the medium, and the concentration after the distribution has taken place is sufficiently high to permit a high rate of respiration only if the concentration of mitochondria is greater than approximately 0.5 mg of protein/ml. Although slower, this distribution occurs also in pH 6.2 saline media in which little or no swelling of the mitochondria takes place, indicating that cytochrome *c* penetrates the intact outer membrane.

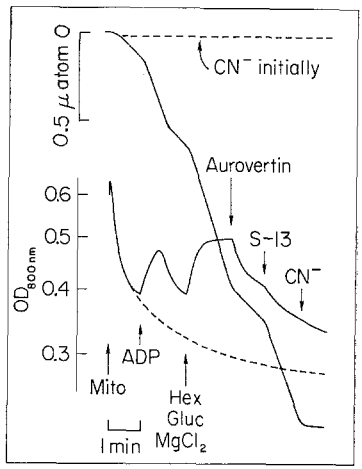

Figure 1. Swelling and contraction of heart mitochondria in 0.1 M NH_4^+ phosphate at pH 6.2. The media contained, in addition, 2.5 mM NH_4^+ malate, 2.5 mM NH_4^+ pyruvate, and 25 μg of cytochrome *c*/ml. The concentrations of the indicated materials were: ADP, 125 μM; hexokinase (Hex), 5 units/ml; glucose (Gluc), 5 mM; $MgCl_2$, 1 mM; aurovertin, 5 nmoles/mg of mitochondrial protein; S-13, 0.5 μM; CN^-, 1 mM.

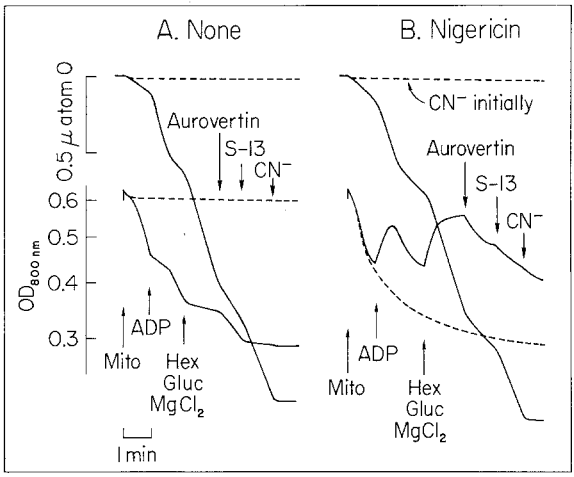

Figure 2. Swelling and contraction of heart mitochondria in 0.1 M K^+ phosphate at pH 6.2. The conditions of incubation were as described in Fig. 1, except that K^+ was substituted for NH_4^+ and 50 nM nigericin was present in part B.

In contrast to liver mitochondria [3], heart mitochondria swell quite rapidly and extensively in NH_4^+ phosphate at pH 6.2 (Fig. 1). Swelling is associated with an increase in the State 4 rate of respiration, and inhibition of respiration has little effect on the swelling other than perhaps increasing its extent slightly. Addition of a small amount of ADP after swelling has taken place initiates a rapid State 3–State 4 respiratory cycle which is paralleled by a contraction–swelling cycle. Subsequent additions of a hexokinase trapping system, aurovertin, and S-13 result in changes which clearly indicate that the contraction is due to net removal of internal P_i by way of oxidative phosphorylation of external ADP.

Substitution of K^+ for NH_4^+ in the system of Fig. 1 yields essentially identical results in regard to respiratory activity, but not to mitochondrial volume changes (Fig. 2A). In the K^+ system, swelling is more extensive and largely energy dependent. Initiation of oxidative phosphorylation results only in a slowing of the swelling, and addition of an uncoupler stops the swelling. However, inclusion of nigericin in the K^+ system renders it essentially equal to the NH_4^+ system in respect to both respiratory activity

and volume changes (Fig. 2B). Similar results are obtained when Na^+ is substituted for NH_4^+.

Addition of mersalyl to respiring heart mitochondria which have been allowed to swell in NH_4^+ phosphate at low pH results in a slow, energy-dependent contraction which is little affected by oligomycin, atractyloside, and similar inhibitors of oxidative phosphorylation (Fig. 3A). Subsequent addition of ADP results in a rapid, oligomycin-sensitive contraction which soon results in the swelling being largely reversed. Addition of an uncoupler after the contraction is complete results in rapid swelling. This swelling can be prevented by prior addition of either oligomycin or atractyloside, indicating that it results from hydrolysis of the ATP formed during the contraction phase [23]. Substitution of arsenate for P_i in the system of Fig. 3A yields similar results in regard to the initial swelling and the subsequent contraction; however, the ADP-induced contraction, al-

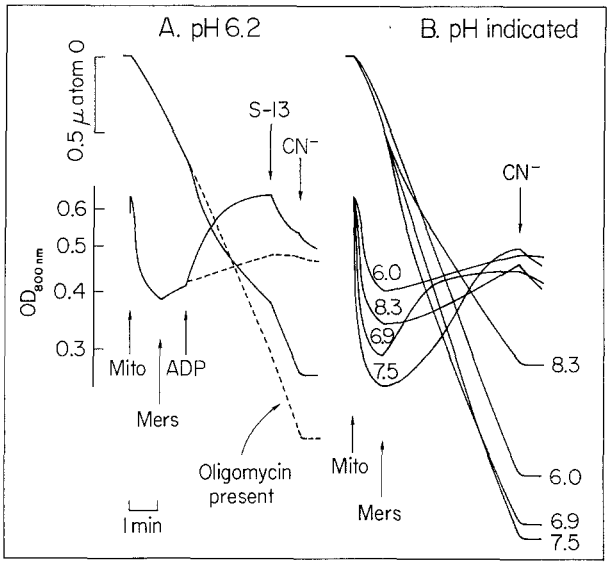

Figure 3. Mersalyl-induced contraction of heart mitochondria in 0.1 M NH_4^+ phosphate as a function of pH. The media contained, in addition, 5 mM NH_4^+ succinate, 3 nmoles of rotenone/mg of mitochondrial protein, and 25 μg of cytochrome *c*/ml. The concentrations of the indicated materials added were: mersalyl (Mers), 5 μM; ADP, 125 μM; S-13, 0.5 μM; CN^-, 1 mM; oligomycin, 5 nmoles/mg of mitochondrial protein.

though sensitive to oligomycin and atractyloside, is considerably less rapid. The observations appear to be generally consistent with the suggestion of Bertagnolli and Hanson [24], based on chemical analyses and low-amplitude swelling and contraction changes of corn mitochondria suspended in sucrose, that mitochondria are capable of ejecting arsenate by arsenylating internal ADP via the phosphorylation pathway and exchanging some of the product for external ADP.

As shown in Fig. 3B, the mersalyl-induced contraction, as well as the swelling that precedes it, is highly pH dependent. It was often observed in experiments similar to that of Fig. 3B that energized mitochondria suspended at alkaline pH, after undergoing an initial rapid swelling upon addition of the mitochondria to the incubation medium, undergo a slow but often extensive energy-dependent contraction without the aid of mersalyl. Since mersalyl inactivates the P_i carrier in respect to both P_i influx and P_i efflux [23], the principal effect of mersalyl thus seems to be simply to inhibit the influx of P_i via the carrier, allowing net efflux of P_i by a mechanism which does not involve the carrier. The existence of this mechanism can be better seen from Fig. 4, which presents the results of two experiments in which the resistance to swelling of heart mitochondria in NH_4^+ phosphate over a period of 1 min was determined as a function of pH of the incubation mixture, energy status of the mitochondria, and presence and absence of ADP and mersalyl. Although different Krebs cycle substrates were used in these experiments, essentially the same results were obtained in respect to resistance to swelling, indicating that the observed effects of pH on the energy-dependent resistance to swelling likely did not result from direct effects of pH on respiration, except perhaps at the extremes of pH.

The experiments of Fig. 4 show that swelling in the absence of mersalyl is most rapid at pH approximately 7.5. Deenergization of the mitochondria with the uncoupler S-13 inhibits swelling slightly at pH below the optimum for swelling and stimulates swelling above the optimum. Assuming "resistance to swelling" to be equivalent to "potential for contraction," the data of Fig. 4 obtained with mitochondria incubated in the absence of mersalyl suggest that the contractile process involved in the phosphorylation-dependent contraction of Figs. 1 and 2B occurs optimally at pH 6.0, and that the contractile process involved in the mersalyl-induced contraction of Fig. 3 occurs optimally at pH 8.5 to 9.0. As can be seen from Fig. 3B, the pH optimum for the high-pH contractile process appears to be considerably lower than pH 8.5 to 9.0 when P_i influx is inhibited with mersalyl.

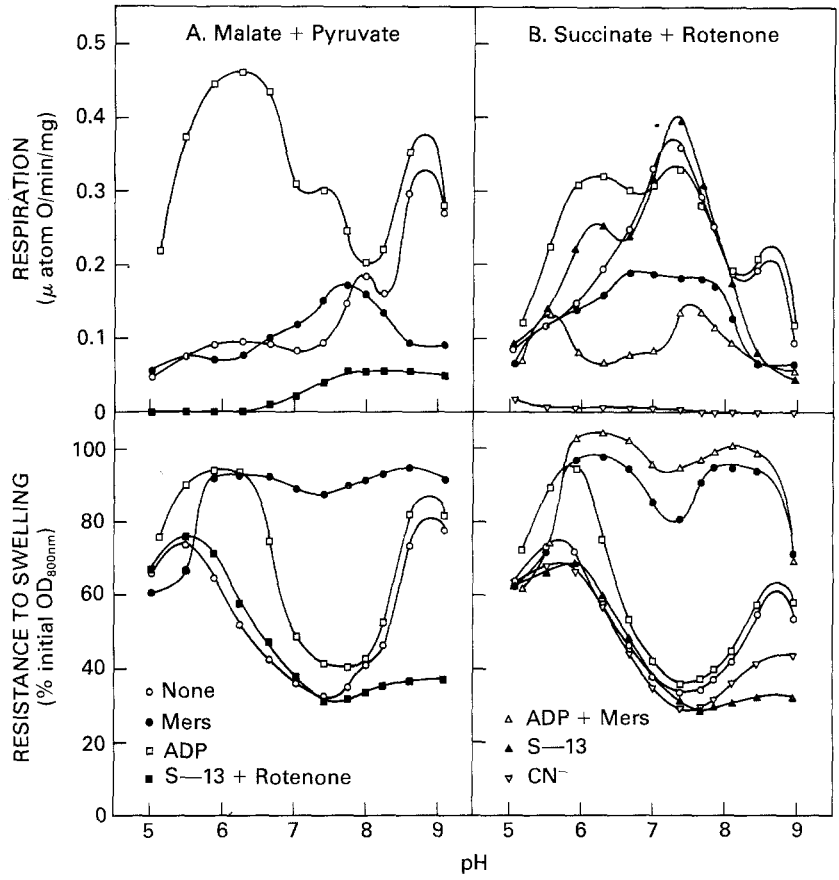

Figure 4. Effects of pH and metabolic status on the swelling of heart mitochondria in 0.1 M NH_4^+ phosphate. All the media contained 25 μg of cytochrome *c*/ml. The concentrations of the indicated materials added were: NH_4^+ malate, 2.5 mM; NH_4^+ pyruvate, 2.5 mM; NH_4^+ succinate, 5 mM; rotenone, 4 nmoles/mg of mitochondrial protein; ADP, 1 mM; mersalyl (Mers), 50 μM (A), 25 μM (B); CN^-, 1 mM; S-13, 0.25 μM (A), 1 μM (B). The values for resistance to swelling were obtained by dividing the OD observed after 1 min of incubation by the initial OD and multiplying the result by 100. The respiration values represent the average rate over a 30-sec period beginning at 45 sec after initiation of incubation. Parts A and B represent different mitochondrial preparations.

Discussion

It is generally accepted that mitochondrial volume changes of the type described here occur in consequence of changes in the osmotic pressure of the matrix due to ion movements across the inner membrane. Considerable success has been achieved in explaining these movements in terms of the chemiosmotic hypothesis, a version of which is summarized in Fig. 5A.

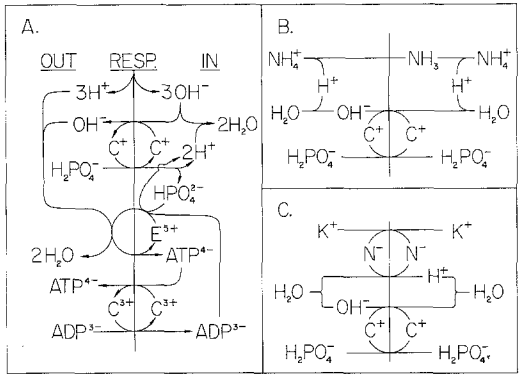

Figure 5. Schemes summarizing (A) chemiosmotic coupling of respiration to phosphorylation, (B) mechanism by which NH_4^+ phosphate equilibrates across the inner mitochondrial membrane (2), and (C) mechanism by which nigericin catalyzes the equilibration of K^+ phosphate across the inner membrane [7, 8]. Nigericin (N) and the C's are exchange-diffusion carriers which can permeate the hydrophobic ion barrier of the inner membrane only when loaded with substrate, and E is the substrate-binding component of the ATPase which catalyzes the phosphorylation of ADP. Phosphate transport is assumed to occur as suggested by Chappell and Crofts [1], and adenine nucleotide transport as suggested by Pfaff and Klingenberg [27]. The number of external protons utilized in the energy-consuming steps per ATP formed is assumed to be that suggested by Brand and Lehninger [28]. The phosphorylation step is assumed to occur by a mechanism similar to that suggested by Kozlov and Skulachev [29]. The substrate-binding component of the ATPase is assumed to possess a 5^+ charge which traps it at the inner surface of the inner membrane. Upon binding ADP^{3-} and HPO_4^{2-} (the binding of HPO_4^{2-} resulting in the proton being released), the component becomes negatively charged and mobile and is propelled by the membrane potential to the outer side of the membrane, where ATP formation takes place. Upon the formation of ATP, the component becomes positively charged and is propelled to the inner side of the membrane, where transfer of the ATP to the aqueous phase takes place.

As will be pointed out below, the observations of the present study can also be explained satisfactorily in terms of this hypothesis.

The passive swelling of mitochondria in NH_4^+ phosphate and K^+ phosphate + nigericin has been well explained in terms of the mechanisms depicted in Figs. 5B [2] and 5C [7, 8]. Under conditions of low pH, respiration in the absence of phosphorylation has little effect on this swelling. Since influx of the salts would be influenced primarily by ΔpH and the influx would likely be sufficiently rapid to maintain ΔpH very low, this appears to be consistent with the chemiosmotic hypothesis. Respiration would be limited primarily by $\Delta\Psi$, which in the absence of ADP or uncoupler might be expected to be very high and to bring about some electrophoresis of external H^+ and NH_4^+ or K^+ into the matrix. This could account for the observed high initial State 4 rates of respiration in these media. Concomitant electrophoresis of anions out of the mitochondria might also be expected and could explain the respiration-dependent inhibition of swelling in nonphosphorylating mitochondria evident in Figs. 1, 2B, and 4, and the mersalyl-induced contraction of nonphosphorylating mitochondria shown in Fig. 3.

The ADP-induced contraction (Figs. 1 and 2B) obviously occurs through removal of internal P_i by way of oxidative phosphorylation of the added (external) ADP. From the chemiosmotic model, we might expect that initiation of this process in respiring mitochondria which have been allowed to swell in NH_4^+ phosphate or K^+ phosphate + nigericin would result in a decrease in $\Delta\Psi$ and, since respiration would likely be controlled primarily by $\Delta\Psi$ before the initiation, in an increase in ΔpH through an increase in matrix pH. The increase in matrix pH would stimulate P_i influx via the carrier and thus would compensate to some extent for the removal of P_i. On the other hand, the increase would promote efflux of the cations (Figs. 5B and 5C). The observed contraction upon initiation of oxidative phosphorylation (Figs. 1 and 2B) can be explained by assuming that the rate of P_i removal exceeds the rate of P_i influx. The contraction proceeds only to the point at which the rates of the two processes become equal, and does not go to completion because the rate of P_i removal decreases and the rate of P_i influx increases as the concentration of internal P_i decreases. The addition of mersalyl (Fig. 3A) results in inhibition of P_i influx and thereby in more extensive removal of internal P_i.

According to this interpretation, the low apparent pH optimum of the phosphorylation-dependent contractile process in extremely swollen mitochondria incubated in the absence of mersalyl (Fig. 4) results from P_i removal being particularly rapid relative to P_i influx at low pH. Since the

rates of the two processes were not measured, nothing certain can be said as to whether this might be due to P_i influx being particularly slow at low pH or to P_i removal being particularly rapid at low pH. However, the close correspondence of the pH optimum for phosphorylation-dependent contraction to the acid pH optimum for resistance to swelling of nonphosphorylating mitochondria suspended in NH_4^+ phosphate in the absence of mersalyl (Fig. 4) suggests that the low pH optimum for the contraction is due to P_i influx being particularly slow at low pH.

The relatively slow net flow of P_i into heart mitochondria at low pH appears to result from a limitation on the entry of the salt at the level of the P_i carrier. Thus, substituting P_i with acetate, which rapidly permeates the inner membrane as the free acid [1], results in rapid and extensive swelling of nonenergized mitochondria throughout the pH range examined in this study, and substituting NH_4^+ with imidazolH^+, which permeates the inner membrane in the same manner as NH_4^+ (Fig. 5B), has no effect on swelling in the acid pH range. Mitchell and Moyle [3] have made observations similar to these with rat liver mitochondria and have suggested that the P_i carrier has an alkaline pH optimum. This explanation seems to be inconsistent with the observations of the present study, because the swelling of heart mitochondria at low pH is initially quite rapid and becomes very slow long before it is complete (Figs. 1 and 3). A better explanation might be that conditions of P_i carrier substrate concentration are relatively unfavorable for net influx of P_i at low pH. Since under the conditions used here (100 mM P_i), carriers with their P_i binding sites at the external surface of the inner membrane likely would be virtually saturated with P_i throughout the examined pH range below the pH optimum for swelling despite wide variation of the concentration of $H_2PO_4^-$, explanation of the pH effect on this basis may be limited to consideration of the concentrations of substrates in the matrix. Assuming the pH to be the same inside the mitochondria as outside, as would tend to be the case (Fig. 5B), particularly when an uncoupler is present, the conditions at low pH would be relatively unfavorable for net influx of P_i. Thus, inside the mitochondria the concentration of OH^- would be low and the proportion of P_i as $H_2PO_4^-$ would be high, conditions favorable for return of entering P_i to the suspension medium. At higher pH the concentration of OH^- would be relatively high and the proportion of P_i as $H_2PO_4^-$ relatively low, conditions relatively unfavorable for return of entering P_i to the suspension medium.

The decrease in the rate of swelling of nonphosphorylating mitochondria as the pH of the medium is increased beyond the optimum for swelling (Fig. 4) might be due to the concentration of external $H_2PO_4^-$ decreasing

significantly beyond the point of virtual saturation of the P_i carrier and to the onset of significant competitive inhibition of the binding of external $H_2PO_4^-$ to the carrier by external OH^-. Some of the swelling that occurs above the optimum is likely due to unmediated entry of P_i. It has been established that the permeability of the inner membrane to anions increases markedly as the pH of the suspension medium is increased beyond neutrality [4, 30], and that this change is associated with an increase in the ability of mitochondria to undergo passive swelling and subsequent energy-linked contraction in NH_4^+ salts [31]. The high apparent pH optimum for energy-dependent contraction of nonphosphorylating mitochondria incubated in the absence of mersalyl (Fig. 4) likely comes about as a result of this relatively high permeability of the inner membrane at high pH and of a consequent relatively rapid electrophoretic extrusion of internal P_i anions. There is some evidence [32] that the permeability of the inner membrane to anions increases also in response to increasing the pH of the matrix. If, as might be expected (Fig. 5A) when $\Delta\Psi$ consumption through electrophoresis of cations into or anions out of the mitochondria is occurring, the internal pH of respiring, nonphosphorylating mitochondria increases substantially as a result of mersalyl inhibition of P_i influx via the carrier, this could account for the relatively low apparent pH optimum for the mersalyl-induced contraction of Fig. 3B.

The effects of the uncoupler S-13 on the swelling and contraction phenomena observed in this study seem to be generally consistent with the above interpretations and the view that it uncouples by mediating H^+ transfer across the inner membrane. In the case of respiring, nonphosphorylating mitochondria, the large enhancing effect of S-13 on swelling in NH_4^+ phosphate at high pH (Fig. 4B) was likely due primarily to the uncoupler eliminating the energy-dependent extrusion of P_i anions. The ability of the uncoupler to increase the rate of swelling of nonrespiring mitochondria in this medium at high pH, evident in Fig. 4, can be explained by assuming that the swelling in the presence of the uncoupler was due in part to unmediated entry of P_i as a result of the high permeability of the inner membrane to anions under these conditions. Unmediated entry of P_i anions would be electrogenic and, as previous studies on the swelling and contraction of heart mitochondria in NH_4^+ salts of strong acids have shown [31], is strongly stimulated by an uncoupler, presumably as a result of the uncoupler transporting the H^+ needed for the equivalent of NH_4^+ influx and electrical neutralization of the permeating anions. The slight inhibitory effect of S-13 on the swelling of nonphosphorylating mitochondria in NH_4^+ phosphate at low pH (Fig. 4), where P_i influx is largely mediated, might

be explained by assuming that the uncoupler decreased the magnitudes of a small increase in matrix pH and a consequent small increase in the rate of P_i influx which came about as a result of NH_3 influx being much more rapid than P_i influx.

Acknowledgments

We thank Dr. H. R. Blackwell for generously providing us with laboratory facilities in the Institute for Research in Vision, and Melissa Richardson and Karen Willardson for technical and secretarial assistance. This work was supported by grants from the Rosenstiel Foundation, the Central Ohio Heart Chapter, Inc., and the U.S. Public Health Service (Research Grant HL 18038 from the National Institutes of Health).

References

1. J. B. Chappell and A. R. Crofts, in: *Regulation of Metabolic Processes in Mitochondria,* J. M. Tager, S. Papa, E. Quagliarello, and E. C. Slater, eds., Elsevier, Amsterdam (1966), p. 293.
2. J. B. Chappell, *Br. Med. Bull.*, **24** (1968) 150.
3. P. Mitchell and J. Moyle, *Eur. J. Biochem.*, **9** (1969) 149.
4. G. P. Brierley, M. Jurkowitz, K. M. Scott, and A. J. Merola, *J. Biol. Chem.*, **245** (1970) 5404.
5. G. P. Brierley, M. Jurkowitz, K. M. Scott, and A. J. Merola, *Arch. Biochem. Biophys.*, **147** (1971) 545.
6. G. P. Brierley, *Mol. Cell. Biochem.*, **10** (1976) 41.
7. B. C. Pressman, E. J. Harris, W. S. Jagger, and J. H. Johnson, *Proc. Natl. Acad. Sci. U.S.A.*, **58** (1967) 1949.
8. E. C. Slater, in: *The Energy Level and Metabolic Control in Mitochondria,* S. Papa, J. M. Tager, E. Quagliarello, and E. C. Slater, eds., Adriatica Editrice, Bari (1969), p. 317.
9. G. F. Azzone, S. Massari, and T. Pozzan, *Biochim. Biophys. Acta,* **423** (1976) 15.
10. A. L. Lehninger, *Physiol. Rev.*, **42** (1962) 467.
11. W. S. Lynn, Jr., S. Fortney, and R. H. Brown, *J. Cell Biol.*, **23** (1964) 9.
12. A. R. Crofts and J. B. Chappell, *Biochem. J.*, **95** (1965) 387.
13. C. D. Stoner and J. B. Hanson, *Plant Physiol.*, **41** (1966) 255.
14. G. F. Azzone, A. Massari, and T. Pozzan, *Biochim. Biophys. Acta,* **423** (1976) 27.
15. L. Packer, *J. Biol. Chem.*, **236** (1961) 214.

16. C. R. Hackenbrock, *J. Cell Biol.*, **30** (1966) 269.
17. J. B. Hanson, B. L. Bertagnolli, and W. D. Shepherd, *Plant Physiol.*, **50** 1972) 347.
18. A. Fonyó, E. Ligeti, and H. Baum, *J. Bioenerg. Biomemb.*, **9** (1977) 213.
19. C. D. Stoner and H. D. Sirak, *Fed. Proc. Abs.*, **30** (1971) 1237.
20. C. D. Stoner, M. M. Ressalat, and H. D. Sirak, *Circ. Res.*, **23** (1968) 87.
21. C. D. Stoner and H. D. Sirak, *J. Cell Biol.*, **56** (1973) 51.
22. C. D. Stoner and H. D. Sirak, *J. Cell Biol.*, **43** (1969) 521.
23. D. D. Tyler, *Biochem. J.*, **111** (1969) 665.
24. B. L. Bertagnolli and J. B. Hanson, *Plant Physiol.*, **52** (1973) 431.
25. P. Mitchell, *Biol. Rev.*, **41** (1966) 445.
26. G. D. Greville, in: *Current Topics in Bioenergetics,* D. R. Sanadi, ed., Academic Press, New York (1969), p. 1.
27. E. Pfaff and M. Klingenberg, *Eur. J. Biochem.*, **6** (1968) 66.
28. M. D. Brand and A. L. Lehninger, *Proc. Natl. Acad. Sci. U.S.A.*, **74** (1977) 1955.
29. I. A. Kozlov and V. P. Skulachev, *Biochim. Biophys. Acta,* **463** (1977) 29.
30. A. Azzi and G. F. Azzone, *Biochim. Biophys. Acta,* **131** (1967) 468.
31. G. P. Brierley and C. D. Stoner, *Biochemistry,* **9** (1970) 708.
32. G. P. Brierley, *Biochemistry,* **9** (1970) 697.