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The Action of Valinomycin in Uncoupling Corn Mitochondria[1]

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ABSTRACT

Valinomycin in the presence of potassium is a potent uncoupler of corn (*Zea mays* L.) mitochondria, eliminating respiratory control. Valinomycin produces higher steady state potassium phosphate swelling which can be reversed to give active shrinkage if mersalyl is added to block the Pi^-/OH^- antiporter. Respiration declines concurrently. Uncouplers accelerate the shrinkage and restore the respiration. The same results can be obtained with sodium phosphate if gramicidin D is substituted as ionophore.

It is concluded that valinomycin uncoupling is the result of cyclic salt transport, with influx pumping of potassium phosphate via the Pi^-/OH^- antiporter and efflux pumping via a K^+/H^+ antiporter. The result is a higher level of steady state swelling, rapid turnover of the proton gradient, and uncoupled respiration rates. The level of steady state swelling can be manipulated by varying the valinomycin or K^+ concentrations, with high concentrations favoring activation of the efflux pump.

A mosaic membrane model with high resistance for proton and monovalent cation penetration to the cation$^+/H^+$ antiporter is used to explain the results.

Valinomycin is widely used as a lipid-soluble, potassium-binding ionophore for rapidly equilibrating the electrochemical potential of K^+ across membranes. With animal mitochondria this equilibration does not effectively uncouple respiration unless an anion (*e.g.*, phosphate or acetate) is present which can be transported at the expense of a proton gradient, in which case there is active swelling (19, 20). If permeability to chloride is induced, respiration is released by Val[2] in active KCl extrusion (3). With low K^+ concentrations, Val does not eliminate respiratory control or phosphorylation of liver mitochondria (11, 17, 21).

Plant mitochondria are at least quantitatively different. There is rapid passive swelling in KCl at neutral pH which is accelerated by gramicidin D (15, 27) or Val (13, 25), demonstrating permeability to chloride which is rate limited by accompanying cation influx. Efflux pumping of the KCl with concomitant shrinkage is not uncoupled by Val although respiration is accelerated (13). With sucrose or mannitol-supported media and low concentrations of K^+, Val or gramicidin will uncouple respiration and eliminate respiratory control with ADP (9, 13, 15, 26). As in animal mitochondria (6), Val plus K^+ will activate plant mitochondrial ATPase, being fully as effective as 2,4-dinitrophenol (12). To an impressive degree, Val plus K^+ produces uncoupling of plant mitochondria resembling that obtained with the classical, proton-conducting uncouplers.

Rat liver (18) and beef heart (4) mitochondria are believed to possess antiporters for exchanging Na^+ for H^+, with much less activity in K^+/H^+ exchange. It has been suggested (P. Mitchell, private communication) that the release of respiration in corn mitochondria with little swelling (9) could arise from the activity of a K^+/H^+ antiporter, and that such an antiporter may be characteristic of plants. It is characteristic of plants that Na^+ is not an essential element (some halophytes excepted), and it is reasonable to speculate that an active K^+/H^+ antiporter exists.

We report here an investigation of Val-uncoupled respiration of corn mitochondria as related to influx and efflux pumping of phosphate salts. (The term "pumping" is used to denote energy-linked salt transport in or out of the matrix.) It seems that a K^+/H^+ antiporter may in fact exist, but that it is not really K^+ specific. Specificity seems to lie primarily with a lipid barrier which has high resistance to cation penetration unless ionophores are used. Uncouplers in turn may promote penetration of protons to this cation$^+/H^+$ antiporter.

MATERIALS AND METHODS

Corn mitochondria (*Zea mays* L., WF9-Tms × M14) were isolated as previously described (8) in 0.4 M sucrose, 50 mM KH_2PO_4, 5 mM EGTA, adjusted to pH 7.6 with tris. In certain cases endogenous K^+ was reduced by substituting NaH_2PO_4 as buffer; this lowered the endogenous K^+ from 172 to 78 nmoles/mg of protein. Potassium was determined by flame emission spectroscopy on 5% trichloroacetic acid extracts.

Reactions were run in 4-ml volumes at 29 C with simultaneous recordings of O_2 and per cent transmission as previously described (8, 9), except that full scale transmittance was set from 20 to 40%. Light transmission reflects the swelling and shrinkage accompanying the uptake and loss of osmotically active solutes (2, 9, 13, 26).

The basic reaction medium was 200 mM sucrose, 1 mM $MgSO_4$, 1 mg/ml BSA, and 10 mM TES buffer adjusted to pH 7.6 with KOH, or NaOH in experiments in which K^+ was a controlled variable. Mitochondrial protein was about 1 mg/vessel. Except for experiments involving measurements of respiratory control (Fig. 1), 1 μg/ml of oligomycin and 15 μM rotenone were included in the basal medium to block the coupling ATPase and any endogenous respiration. Oligomycin slightly promotes active phosphate and arsenate uptake (2) and eliminates the respiratory stimulation due to phosphate

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[2] Abbreviations: Val: valinomycin; EGTA: ethyleneglycol-bis-(β aminoethyl ether)-N,N'-tetraacetic acid; FCCP: *p*-trifluoromethoxy(carbonyl cyanide)phenyl hydrazone; Mers: mersalyl; DTE: dithioerythritol.

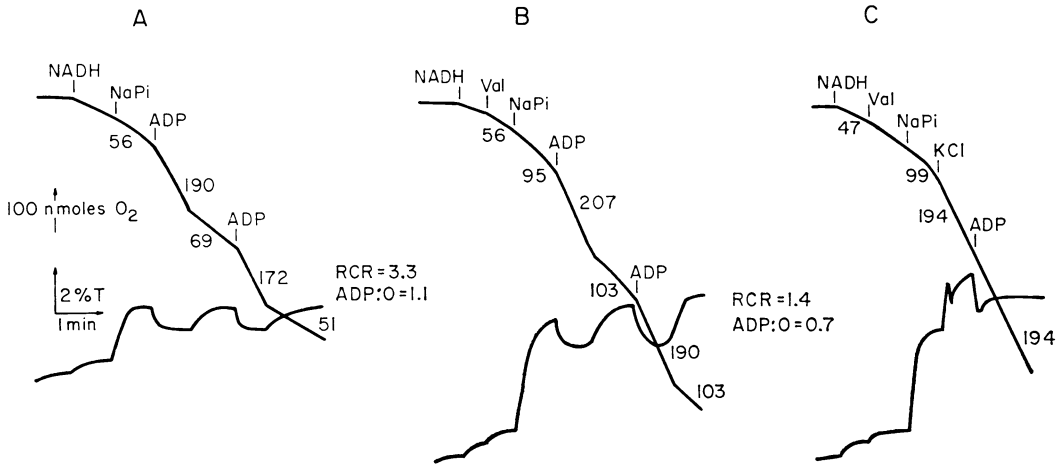

FIG. 1. Uncoupling of corn mitochondria by valinomycin. Basic medium of 200 mM sucrose, 1 mM $MgSO_4$, 1 mg/ml of BSA, and 10 mM TES buffer (pH 7.6) at 29 C, containing 1.1 mg/ml of mitochondrial protein, with additions of 2 μmoles of NADH, 10 mM NaH_2PO_4, 300 nmoles of ADP, 0.17 μg of Val, or 1 mM KCl as indicated. Numbers are nmoles O_2/min·mg protein. A: Control; B: effect of Val with endogenous K^+; C: effect of Val with added KCl.

turnover at coupling sites (10). Phosphate was added as the K^+ or Na^+ salt to 10 mM final concentration. Exogenous NADH is oxidized at the outer surface of the inner membrane of plant mitochondria subsequent to the rotenone-sensitive site (7), and was used as substrate to eliminate problems of uncoupling substrate acid transport into the matrix (unpublished observations). Average ADP/O and respiratory control ratios for NADH were 1.2 and 3.1, respectively.

RESULTS

Valinomycin Uncoupling. Uncoupling and loss of respiratory control caused by Val are shown in Figure 1. The only K^+ present in Figure 1, A and B, was that endogenous to the mitochondria, 172 nmoles/mg of protein. However, in the presence of Val, this endogenous K^+ was adequate to produce more extensive phosphate swelling upon addition of NaH_2PO_4, and to lower the respiratory control and ADP-O ratios (*cf.* parts A and B of Fig. 1). The gradual increase in respiration after addition of NaH_2PO_4 probably reflects the gradual release of endogenous K^+. If additional K^+ is added, the loss of respiratory control is complete (Fig. 1C). The swelling trace, which normally shows a sharp contraction on addition of ADP and reswelling when the ADP is exhausted (2, 8, 9), is altered in the reswelling phase by Val and 1 mM K^+ (*cf.* parts A and C of Fig. 1). Uncoupling of respiration reaches a maximum at 3 mM K^+ (data not shown).

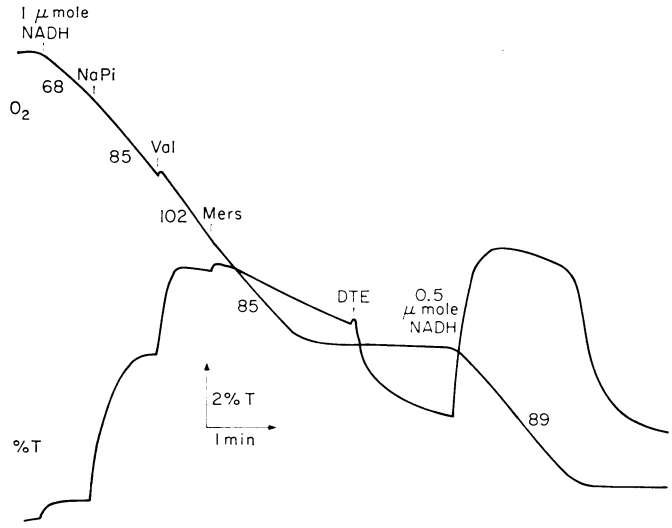

FIG. 2. Inhibition of phosphate efflux and shrinkage by Mers, and reversal with DTE. Basic medium (Fig. 1) plus 1 μg/ml of oligomycin and 15 μM rotenone. Additions were of 10 mM NaH_2PO_4, 0.35 μg of Val, 25 μM Mers. and 0.5 mM DTE.

Influx and Efflux Pumping of Phosphate. It will be noticed in Figure 1, B and C, that Val does not produce continuous swelling. Rather, a new and higher steady state of swelling is attained. This may reflect increased influx pumping of potassium phosphate which is offset at steady state by passive back leakage. An alternative explanation is that steady state represents equilibrium between active influx pumping and active efflux pumping, both of which could contribute to uncoupling. Corn mitochondria are active in both influx pumping of salts of "permeant" anions (*e.g.*, phosphate and acetate), and efflux pumping of salts of anions derived from strong acids such as chloride and nitrate (10, 26). Val accelerates respiration in both cases (13).

Resolution of these alternatives was attempted by using Mers to block the Pi^-/OH^- antiporter (24). In corn mitochondria, Mers strongly inhibits the influx pumping and passive efflux of phosphate, and the inhibition can be reversed by sulfhydryl-protecting reagents (2, 9). In the present experiments we often found that 25 μM Mers added to mitochondria heavily loaded with phosphate would induce some passive shrinkage. An extreme example is given in Figure 2. In these cases the mercurial may be causing some permeability change (4), for the shrinkage is independent of respiration. DTE is an effective agent in restoring phosphate fluxes.

If Mers is added to K-phosphate-loaded mitochondria to block further movement via the Pi^-/OH^- antiporter, subsequent addition of Val produces shrinkage (Fig. 3A). The shrinkage is active, depending on respiration (Fig. 3B).

Figure 4A shows the result of adding Mers after Val, using K^+-depleted mitochondria (78 nmoles K^+/mg of protein). Endogenous K^+ is adequate to produce only a trace of additional swelling on addition of Val, and active shrinkage after Mers is trivial. However, with 1 mM K^+ present Val produces a high steady state level of swelling, and blocking the Pi transporter immediately switches the mitochondria to net efflux pumping (Fig. 4B). Respiration declines (see also Fig. 3). Presumably

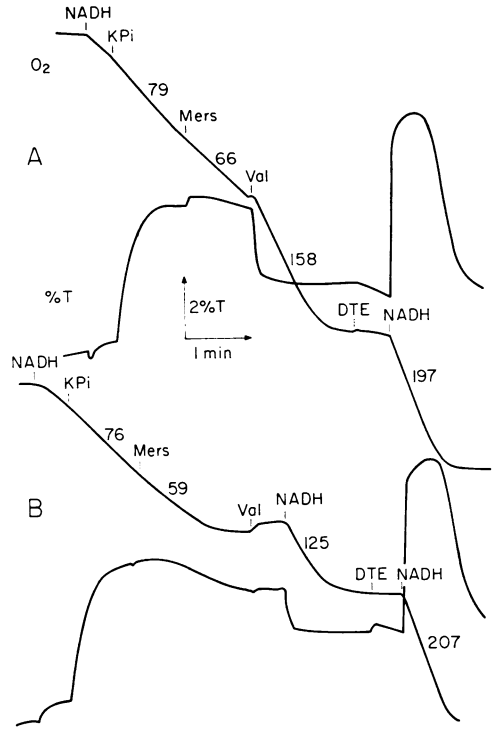

FIG. 3. Respiration-dependent shrinkage induced in phosphate-loaded, Mers-blocked corn mitochondria by Val. Medium and additives as in Figure 2, but substituting 1.5 μmoles of NADH and 10 mM KH_2PO_4; NADH additions in trace B were 0.5 μmole, 0.25 μmole, and 0.5 μmole.

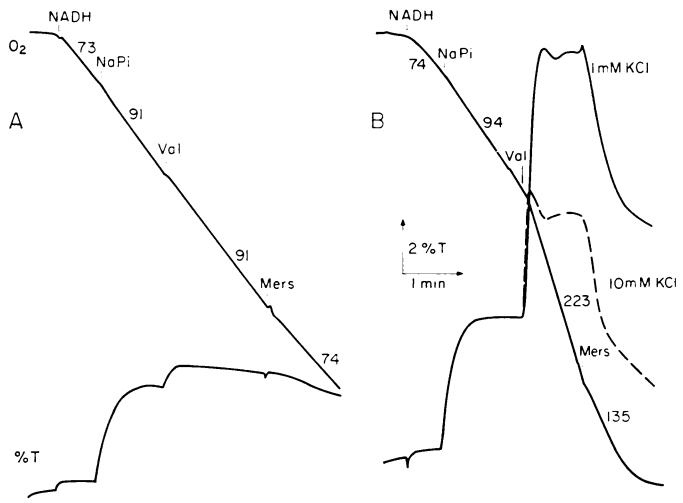

FIG. 4. Induction of shrinkage by Mers in phosphate-swollen mitochondria. Basic medium plus oligomycin and rotenone and additives as in Figure 2, but using low K^+ mitochondria and 1.5 μmoles of NADH. A: No added K^+; B: initial addition of 1 mM KCl (broken line represents 10 mM KCl from a parallel determination with the same mitochondria).

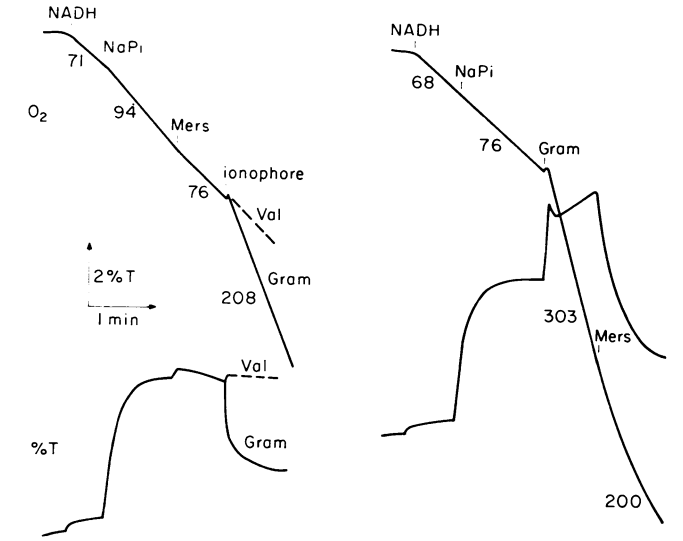

FIG. 5. Respiration-linked shrinkage of sodium phosphate-loaded, Mers-blocked mitochondria by gramicidin D. Medium, additives, and low K^+ mitochondria were as in Figure 4, with 5 μg of gramicidin D as indicated. Val at 0.35 μg had no effect (broken lines, from parallel determination with the same mitochondria).

the respiration inhibited by Mers is that maintained by action of the Pi^-/OH^- antiporter, and the proposed H^+/K^+ antiporter (16, 18) must account for most of the respiration after Mers. (The level of respiration maintained by "proton leak" would be that after addition of NADH.)

If the added K^+ is increased to 10 mM there is a sharp oscillation in the swelling curve, and a lower steady state swelling is obtained (Fig. 4B). Addition of Mers induces proportionately greater efflux pumping and shrinkage. Maximum steady state swelling under the conditions of Figure 4B is produced with 1 mM KCl; the decline with high concentrations levels out at 8 to 10 mM (data not shown).

It appears from the foregoing that there is a pathway for efflux of phosphate from the mitochondria in addition to the Pi^-/OH^- antiporter. This pathway has high resistance such that phosphate efflux becomes significant only in association with respiration-driven K^+ efflux facilitated by Val. Thus the augmented steady state swelling in the presence of K^+ and Val probably reflects a balance between energy-linked influx of Pi (accompanied passively by K^+) and energy-linked efflux of K^+ (accompanied passively by phosphate). When the K^+ concentration is high (*e.g.*, 10 *versus* 1 mM KCl, Fig. 4B) the efflux pumping becomes relatively more effective and the level of steady state swelling is lowered.

The influx-efflux steady state is not confined to K^+. By substituting gramicidin D for Val the same result can be obtained with sodium phosphate pumping (Fig. 5).

It was found that Val concentration would also affect the steady state swelling (Table I). Val-induced swelling in 10 mM potassium phosphate is maximal at about 0.04 μg of Val/mg of protein, whereas the optimum concentration for active shrinkage after adding Mers is about 0.2 μg/ml of protein (Table I). Respiration is maximally stimulated at this higher concentrataion of Val, and there is maximum depression of respiration by Mers (Table I). This result suggests that influx pumping increases with Val concentration, but that it is offset by a more rapidly rising efflux pump.

Uncouplers and Efflux Pumping. The uncoupler FCCP will also produce a small shrinkage after Mers blocks the Pi transporter, but only if the mitochondria are respiring (*cf.* parts A and B of Fig. 6). Oddly, once respiration has ceased, efflux pumping cannot be reinstituted by simple addition of NADH (Fig. 6B). It is necessary to remove the Mers block, and now the phosphate efflux is passive. Dinitrophenol gives the same result as FCCP (data not shown).

The effect of FCCP in producing shrinkage after the Pi^-/OH^- transporter is blocked is very dramatic in Val-treated mitochondria (Fig. 7A). The abrupt shrinkage can be in-

Table I. *Active Phosphate Swelling, Respiratory Release, and Active Shrinkage of Corn Mitochondria in Response to Added Valinomycin*

Experiments were performed as in Figure 4B but with addition of 10 mM KH_2PO_4 instead of NaH_2PO_4, and omission of KCl. Swelling is $\Delta\%T$ obtained in one minute due to addition of Val, which approximates steady state. Shrinkage induced by addition of Mers is reported as initial rate graphically obtained as the tangent. Mitochondrial protein was 0.9 mg.

Val	Swelling	Shrinkage	Respiration following Additions			
			NADH	Pi	Val	Mers
ng	$\Delta\%T$	$\Delta\%T/min$	*nmoles O_2/min·mg protein*			
7	2.7	1.5	82	101	143	120
17	5.8	2.4	89	114	209	176
35	6.1	5.0	89	111	275	190
70	4.1	7.1	89	113	327	199
175	2.0	8.1	89	116	400	207
350	1.6	7.4	91	115	385	228

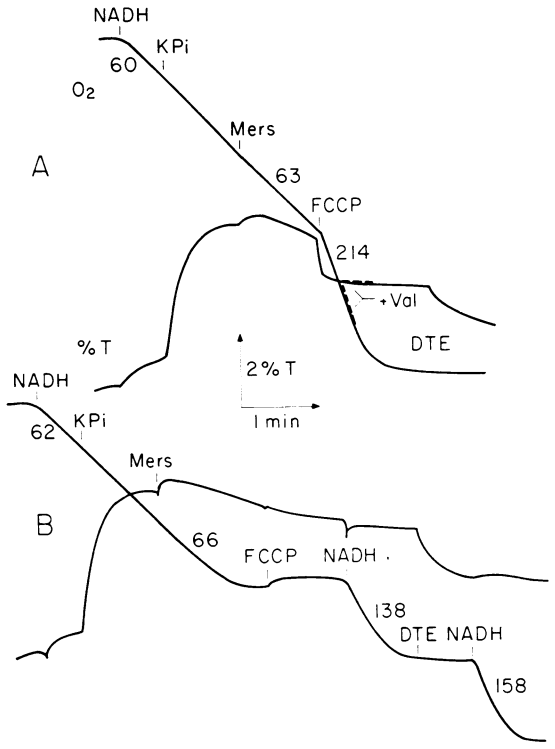

FIG. 6. Respiration-linked shrinkage produced by uncoupling. Basic medium with oligomycin, rotenone, Mers, Val, and DTE was as in Figure 2, but substituting 10 mM KH_2PO_4 and 1 μmole of NADH. Broken lines are from a parallel experiment showing addition of Val had no effect on uncoupled mitochondria. In trace B, NADH additions were 0.5 μmole, 0.25 μmole, and 0.25 μmole.

stituted by uncouplers at any point along the efflux pumping curve; uncoupling does not alter the final level of shrinkage, but only the rate of attaining it. It is unlikely that FCCP under these circumstances is simply making the membrane more permeable to phosphate (the Pi^-/OH^- antiporter is blocked), because Val and respiration are required. The Val-uncoupled respiration which is lost on addition of Mers is recovered by FCCP, which indicates the uncoupler is acting in proton conduction (Fig. 7A), and it is probably through proton conduction that the uncoupler accelerates efflux pumping.

An interaction between FCCP and Val cannot be seen when Na^+ is substituted for K^+ (Fig. 7B). Apparently, the acceleration of shrinkage by FCCP is limited to conditions where the ionophore binds the cation.

DISCUSSION

The question raised here is whether collapse of a proton gradient via a K^+/H^+ antiporter can account for the uncoupling action of Val on corn mitochondria. Without having a specific inhibitor for the presumed K^+/H^+ antiporter, it is difficult to demonstrate its presence directly. In these experiments we have approached the matter indirectly by loading with phosphate salts and then blocking the contribution of the Pi^-/OH^- antiporter with Mers. Any Val-stimulated respiration which remains and which produces active shrinkage (contraction) is presumably attributable to the K^+/H^+ antiporter. Indeed, if one adopts the chemiosmotic view that respiratory chain electron transfer produces only a proton gradient, such an antiporter is required (16).

A suitable chemiosmotic model for examining the data is that of Brierley (4), derived from work with beef heart mitochondria. Figure 8 is a slightly modified model of this type which emphasizes the requirement for passive permeation sites of high resistance—but not as high as in animal mitochondria—and the establishment of steady states between influx and efflux pumping. As drawn, the model separates influx (Fig. 8A) and efflux (Fig. 8B) pumping of phosphate salt as experimentally observed here, and attempts to summarize graphically the fluxes producing steady state osmotic equilibration (Fig. 8C). Figure 8C also includes representation of diffusive influx of KCl down a chloride-concentration gradient, with active efflux pumping and shrinkage from energy provided by respiration (or ATP; ref. 23). In a fluid mosaic model (22), the specific antiporters would be integral proteins, and presumably the nonspecific permeation would be through phospholipid domains of high resistance.

Influx pumping of phosphate is accomplished by the proton motive force driving Pi^-/OH^- exchange with passive penetra-

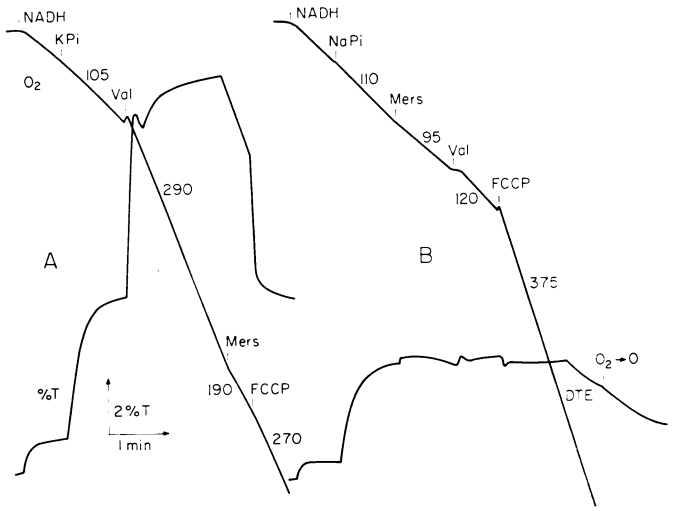

FIG. 7. Acceleration of Val-induced shrinkage by FCCP (A), and ineffectiveness of Val plus FCCP on shrinkage of sodium phosphate-loaded mitochondria (B). Low K^+ mitochondria with medium and additives were as in Figures 2 and 6. Two μmoles of NADH were added.

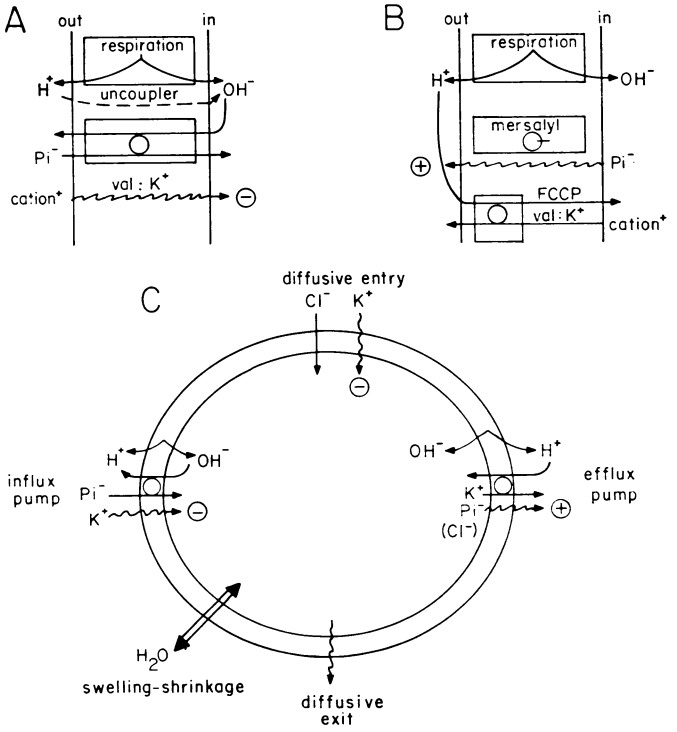

FIG. 8. A chemiosmotic model to explain the Val uncoupling of corn mitochondria, attributable to cyclic salt transport. A: Influx pumping of phosphate with K^+ influx facilitated by Val; B: efflux pumping in phosphate-loaded, Mers-blocked mitochondria, with access to the cation$^+$/H^+ antiporter facilitated by Val and FCCP; C: steady state swelling which can be perturbed by ionophores.

tion of cations down the electrical potential gradient, the latter facilitated by ionophores (Fig. 8A). In efflux pumping, the proton motive force drives K^+/H^+ exchange, and the phosphate fluxes down the potential gradient (Fig. 8B). Collectively, both systems are operative in producing steady state salt concentrations in the matrix with associated osmotic volume adjustment (Fig. 8C).

Valinomycin perturbs the system and steady states are altered (Fig. 4). Low concentrations of Val favor influx pumping, and only after uptake of considerable salt, demonstrated by swelling, does efflux pumping (and to some unknown degree passive leakage) balance the influx. Higher concentrations of Val (Table I) or higher concentrations of K^+ (Fig. 4) are required to maximize the efflux pumping, presumably because of resistance of K^+ entry to the K^+/H^+ antiporter. Experimentally, the existence of the efflux pump was observed only by blocking the Pi^-/OH^- antiporter. Under this circumstance, the influx pathway for collapse of the proton gradient is denied and respiration declines (Fig. 4 and Table I).

Uncouplers may facilitate penetration of H^+ to or from the K^+/H^+ antiporter, explaining the acceleration of efflux pumping (Fig. 7A), and this penetration is suggested in the model (Fig. 8B). The fact that uncoupler-mediated shrinkage is dependent on respiration (Fig. 6) and acts in concert with Val only when K^+ is present (Fig. 7) suggests that the role of uncouplers may be more complex than simple transmembrane conduction of protons through lipid domains; specifically, proton accessibility to enzymes may be involved (1, 9, 14), or permeability changes may be induced (5).

There is no reason to believe that the proton-transporting antiporter is specific for K^+. By substituting gramicidin for Val, the same phenomena can be observed with Na^+ (Fig. 5). A single species of cation$^+$/H^+ exchanging enzyme may function with a number of cations, with degrees of cation specificity (in the absence of ionophore) imposed by the protective lipid in which the exchanger is embedded. This does not preclude some cation binding specificity for the enzyme, but it implies that a primary constraint on its functioning lies in the lipid barrier. An alternative to a selective lipid barrier would be direct participation of the ionophore by binding to the exchange carrier. With high salt concentrations, however, plant mitochondria are very active in salt extrusion and shrinkage without ionophores (13, 23, 27).

As visualized in this discussion, the release of acceptorless respiration and loss of respiratory control in plant mitochondria caused by Val plus K^+ is attributable to augmented cyclic salt transport. We suspect that quantitative distinctions between plant and animal mitochondria with respect to Val uncoupling must lie in anion permeability of the membranes (3). Another distinction may be in the effectiveness of the lipid barrier in discriminating between Na^+ and K^+ in exchange transport.

The model in Figure 8 is based on principles largely laid down by Mitchell (16) in formulating his chemiosmotic hypothesis. It differs primarily in not accepting the postulate that the cations extruded in exchange for H^+ are simply drawn back in collapsing the membrane potential (diagram, p. 119 of ref. 16). This may occur to a small degree, but as discussed above, the shrinkage and decline in respiration on addition of Mers (Fig. 4B) demonstrate that efflux of anion is largely responsible for collapse of the potential. Thus, at steady state (Fig. 4B), the influx of Pi via the Pi^-/OH^- antiporter (Fig. 8A) is clearly contributing to the uncoupled respiration, but because there is no net transport, Pi must be cycling back out. The mechanism for cycling out is that which produces net efflux when Mers is added (Fig. 8B).

LITERATURE CITED

1. AZZONE, G. F. AND S. MASSARI. 1973. Active transport and binding in mitochondria. Biochim. Biophys. Acta 301: 195-226.
2. BERTAGNOLLI, B. L. AND J. B. HANSON. 1973. Functioning of the adenine nucleotide transporter in the arsenate uncoupling of corn mitochondria. Plant Physiol. 52: 431-435.
3. BRIERLEY, G. P. 1970. Energy-linked alteration of the permeability of heart mitochondria to chloride and other anions. Biochemistry 9: 697-707.
4. BRIERLEY, G. P., M. JURKOWITZ, AND K. M. SCOTT. 1973. Ion transport by heart mitochondria. XXVII. The relation of mercurial-dependent adenosine triphosphatase activity to ion movements. Arch. Biochem. Biophys. 159: 742-756.
5. CASWELL, A. H. 1969. Proton permeability and the regulation of potassium permeability in mitochondria by uncoupling agents. J. Membrane Biol. 1: 53-78.
6. CEREIJO-SANTALÓ, R. 1970. Ion movements in mitochondria. *In:* E. E. Bittar, ed., Membranes and Ion Transport, Vol. 2. Wiley-Interscience, New York. pp. 229-258.
7. DONCE, R., C. A. MANNELLA, AND W. D. BONNER, JR. 1973. The external NADH dehydrogenases of intact plant mitochondria. Biochim. Biophys. Acta 292: 105-116.
8. HANSON, J. B. 1972. Ion transport induced by polycations and its relationship to loose coupling of corn mitochondria. Plant Physiol. 49: 707-714.
9. HANSON, J. B., B. L. BERTAGNOLLI, AND W. D. SHEPHERD. 1972. Phosphate-induced stimulation of acceptorless respiration in corn mitochondria. Plant Physiol. 50: 347-354.
10. HANSON, J. B. AND R. J. MILLER. 1967. Evidence for active phosphate transport in maize mitochondria. Proc. Nat. Acad. Sci. U.S.A. 58: 727-734.
11. HÖFER, M. AND B. C. PRESSMAN. 1966. Stimulation of oxidative phosphorylation in mitochondria by potassium in the presence of valinomycin. Biochemistry 5: 3919-3925.
12. JUNG, D. W. 1974. The 2,4-dinitrophenol-stimulated adenosine triphosphatase activity in cauliflower and corn mitochondria. Ph.D. dissertation. University of Illinois, Urbana.
13. KIRK, B. I. AND J. B. HANSON. 1973. The stoichiometry of respiration-driven potassium transport in corn mitochondria. Plant Physiol. 51: 357-362.
14. MASSARI, S. AND G. F. AZZONE. 1970. The mechanism of ion translocation in mitochondria. 2. Active transport and proton pump. Eur. J. Biochem. 12: 310-318.

15. MILLER, R. T., W. S. DUMFORD, AND D. E. KOEPPE. 1970. Effects of gramicidin on corn mitochondria. Plant Physiol. 46: 471- 474.
16. MITCHELL, P. 1966. Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation. Glynn Research Ltd., Bodmin, Cornwall, England.
17. MITCHELL, P. 1969. The chemical and electrical components of the electrochemical potential of H^+ ions across the mitochondrial cristae membrane. *In:* L. Ernster and Z. Drakota, eds., Mitochondria: Structure and Function. Academic Press, New Yok. pp. 219-232.
18. MITCHELL, R. AND J. MOYLE. 1969. Translocation of some anions, cations and acids in rat liver mitochondria. Eur. J. Biochem. 9: 149-153.
19. MOORE, C. AND B. C. PRESSMAN. 1964. Mechanism of action of valinomycin on mitochondria. Biochem. Biophys. Res. Commun. 15: 562-567.
20. OGATA, E. AND H. RASMUSSEN. 1966. Valinomycin and mitochondrial ion transport. Biochemistry 5: 57-66.
21. ROTTENBERG, H. 1970. ATP synthesis and electrical membrane potential in mitochondria. Eur. J. Biochem. 15: 22-28.
22. SINGER, S. J. AND G. L. NICOLSON. 1972. The fluid mosaic model of the structure of cell membranes. Science 175: 720-731.
23. STONER, C. D., T. K. HODGES, AND J. B. HANSON. 1964. Chloramphenicol as an inhibitor of energy-linked processes in maize mitochondria. Nature 203: 258-261.
24. TYLER, D. D. 1969. Evidence of a phosphate-transporter system in the inner membrane of isolated mitochondria. Biochem. J. 111: 665-677.
25. WILSON, R. H., J. DEVER, W. HARPER, AND R. FRY. 1972. The effects of valinomycin on respiration and volume changes in plant mitochondria. Plant Cell Physiol. 13: 1103-1111.
26. WILSON, R. H., J. B. HANSON, AND H. H. MOLLENHAUER. 1969. Active swelling and acetate uptake in corn mitochondria. Biochemistry 8: 1203-1213.
27. YOSHIDA, K. 1968. Swelling and contraction of isolated plant mitochondria. II. Reversible metabolism-dependent contraction. J. Fac. Sci. Univ. Tokyo 10: 63-82.