The Stoichiometry of Respiration-driven Potassium Transport in Corn Mitochondria

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ABSTRACT

Determinations were made for corn (Zea mays L., WF9-Tms × M14) mitochondria of the stoichiometric relationship between K⁺ transport and bond energy produced in respiration (K⁺/~ ratio). With inward pumping of potassium acetate activated by NADH oxidation, the initial rate of K⁺ transported into the sucrose inaccessible space varied between 0.58 and 0.97 K⁺/~, assuming 2 high energy bond equivalents per NADH oxidized. Only small amounts of H⁺ were ejected. Valinomycin did not alter the ratio.

Efflux pumping of K⁺ in KCl media gave a K⁺/~ of 0.92. Substituting sucrose for KCl caused a drop in respiration and H⁺ ejection, indicating a requirement for cation efflux pumping in acceptorless respiration. Valinomycin added to the KCl medium accelerated passive swelling but did not impair subsequent efflux pumping, although respiration was partially uncoupled.

A number of estimates have been made of the energy requirement for cation transport in rat liver mitochondria. Energy is estimated in terms of ATP equivalents, or "~." Influx pumping of K⁺ induced by valinomycin has given K⁺/~ ratios of 3.2 to 7.9 (10, 27, 28). Rossi and Azzone (29) have provided an inverse confirmation of these values by showing that valinomycin-activated efflux of four K⁺ can drive synthesis of one ATP. In the absence of valinomycin, Briereley et al. (6) report beef heart mitochondria to give K⁺/~ ratios of 1.1 to 1.4 in potassium acetate with various substrates and ATP as energy sources. They note that lower respiration rates favor high efficiency, and that valinomycin increases efficiency.

In the presence of phosphate the Ca⁺/~ ratio for liver mitochondria is close to 2 (20), and Sr⁺ gives about the same value (7). A Mn⁺/~ ratio of 1.8 has been reported (9). More recently, however, studies with blowfly mitochondria show Ca⁺/~ ratios of about 0.6 (8). This is of special interest since blowfly mitochondria are like corn mitochondria in having a phosphate requirement for Ca⁺ uptake.

Energy-dependent efflux pumping has been studied in animal mitochondria (1–5, 11, 22) but not in relation to high energy equivalents formed. It is simply shown that salt efflux occurs in association with water loss and contraction.

We are interested in determining the relationship between acceptorless respiration and ion transport for corn mitochondria. Although stoichiometries are suggested to be of limited value due to incomplete coupling (31), ion transport data are required in making any estimates of the role transport plays in respiratory control. With this view in mind, we have determined K⁺/~ ratios for corn mitochondria for both influx and efflux pumping. The ratios approach unity and are thus much lower than for rat liver mitochondria.

MATERIALS AND METHODS

Mitochondria were isolated from 3-day-old etiolated corn shoots (Zea mays L., WF9-Tms × M14) as previously described (19), but with two exceptions: (a) 5 mM EGTA was used in the isolation medium instead of EDTA to minimize removal of Mg⁺ and (b) ADP was omitted from the first sucrose resuspension medium. The final mitochondrial suspension was made in CO₂-free 0.2 mM sucrose to give a protein concentration of 15 to 20 mg/ml. The pH of this suspension, which was routinely 7.1, was adjusted to 7.3 with 0.2 mM tris before use.

Measurement of K⁺ Flux during Active Swelling and Contraction. The reaction mixture consisted of a final concentration of 40 mM potassium acetate and 120 mM sucrose for active swelling studies or 0.1 M KCl for contraction studies, plus 0.5 mM TES adjusted to pH 7.3 with tris, and 1 mg bovine serum albumin/ml. In addition, 2.9 ml of the medium contained 145 μCi of THO (18 × 10⁶ cpm/ml) and 316 μCi of uniformly labeled ¹³C-sucrose (11 × 10⁶ cpm/ml). The medium was oxygenated prior to the experiment. Duplicate aliquots containing 2.9 ml of the reaction mixture were used in each assay, one being placed in a thermostated Plexiglas vessel in the light path of a Beckman B spectrophotometer, the other in a test tube in a constant temperature circulating bath connected to the vessel. Temperatures of the bath were set 0.1 to 0.3 C above room temperature (24.6–26 C), and temperature differences between spectrophotometer vessel and bath did not exceed 0.2 C. The spectrophotometer vessel was stirred with a magnetic stirrer and fitted with an oxygen electrode (Yellow Springs Instrument Co.) and a combination pH electrode (Sargent S-30070-10). Simultaneous recordings were made of O₂ consumption, pH changes, and percentage of transmission at 520 nm.

The experiment was initiated by the simultaneous addition of 0.4 ml of mitochondrial suspension to the reaction mixtures in the spectrophotometer and in the test tube. After ap-

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Abbreviations: EGTA: ethyleneglycol-bis-(α-amino-ethyl-ether)-N,N'-tetraacetic acid; PCA: perchloric acid; THO: tritiated water.
proximately 5 min, during which time passive swelling was occurring, a series of seven 300-μl aliquots from the test tube were layered into microcentrifuge tubes (see below for centrifugal filtration procedure). At 15- or 30-sec intervals 0.33 μmole of NADH (6.5 μl), pH adjusted with HCl to 7.2, were added to and mixed with the mitochondrial mixture in successive centrifuge tubes. No substrate was added to the last sample in the series. This sample represented the mitochondrial contents at the time of addition of NADH; the others 15, 30 sec after NADH addition. The last six samples were then centrifuged together; the first sample to which NADH had been added was reserved for later centrifugation to obtain a sample during mitochondrial recontraction or swelling. At the same time that centrifugation of the series of samples was initiated, 3.6 μmoles of NADH (72 μl) were injected into the spectrophotometer vessel, and absorbance, O₂ consumption, and pH were observed throughout the experiment. After NADH oxidation was complete and when changes in absorbance and pH ceased, aliquots of standard 0.1 N HCl were injected into the vessel for calibration of pH. Calibration showed that the titration curve of the system was linear within the observed pH range.

**Centrifugal Filtration.** The centrifugal filtration technique described by Harris and van Dam (17) and Werkheiser and Bartley (33) was used with a few modifications to obtain samples of mitochondria separated from the reaction medium for analysis of K⁺, THO, υ-C-sucrose, and protein contents. The 300-μl samples of the reaction mixture which were transferred to 400-μl polyethylene centrifuge tubes were carefully layered over 30 μl of silicone fluid, specific gravity, 1.0183 (obtained by mixing Dow Corning 200 and 560 silicone fluids), which in turn had been layered over 40 μl of 1.5 N PCA. Samples were centrifuged at indicated times during the experiment for 1 min in a Beckman/Spinco Model 152 microfuge at 140 v. The supernatant was removed with a syringe, and the polyethylene tube was cut at the level of the silicone fluid. The remaining silicone fluid in the bottom of the tube was removed as carefully as possible, and the PCA extract was transferred to another tube with a 50-μl Hamilton syringe and mixed thoroughly. If this mixing was omitted, a gradient in K⁺ concentration in the PCA extract was observed, decreasing with distance from the protein pellet. Care was taken not to include any of the silicone fluid in the PCA extract. Twenty μl of the PCA extract were used for radioactivity determinations, and 15 μl were used for determinations of K⁺ content.

**Analyses of Centrifuged Samples.** The K⁺ content of mitochondrial samples obtained by the centrifugal filtration method was determined after dilution with 4.0 ml of a standard LiCl solution by emission flame photometry. The protein content of pellets obtained by centrifugal filtration, as well as those of mitochondrial suspensions, was determined by a modification of the Lowry method (21). Radioactivity in PCA extracts was measured by liquid scintillation.

The volume of THO was used as an estimate of total mitochondrial space, and the "υ-C-sucrose accessible space as that portion of the total external to the inner membrane. The inner mitochondrial compartment (sucrease inaccessible or matrix space) was then obtained as the difference between the total space and sucrose accessible space.

**Measurement of K⁺ Activity.** A Beckman cationic glass electrode (No. 39047) was used to monitor continuously potassium ion activity (K⁺) of the medium. The electrode was connected to a Heathkit pH recording electrometer and the calomel cell of a Sargent or Beckman combination pH electrode was used for the reference electrode. Although the combination electrode was also used to measure pH changes simultaneously in the medium, no interaction of the two measurements could be demonstrated. In these experiments the pH electrode was connected to a Beckman Century SS or Zeromatic II pH meter and recordings made on a Sargent DSR recorder.

Calibration of the cationic electrode was performed prior to each experiment by adding aliquots of 1 m KCl to the potassium-free reaction mixture to give a final concentration of 1 mm K⁺. Other substances which were added during these experiments were tested and found not to affect the cationic electrode.

**Determination of Chloride Extrusion during Energy-dependent Contraction.** The method used was similar to that described above except that 1.2 μc of υ-Cl (8.8 × 10⁶ cpm/ml) were included in the reaction mixture containing 0.1 m KCl. THO and υ-C-sucrose were omitted. Mitochondria were added to this medium and while passive swelling occurred five pairs of samples were prepared. To one sample in each pair 0.33 μmole of NADH was added, and the mitochondria were allowed to contract for 1 min prior to centrifuging the two samples together. The total time from the addition of mitochondria to centrifugation ranged from 12 to 17 min for the five pairs. Potassium, υ-Cl, and protein contents were determined on the PCA extract of the pellet obtained from centrifugal filtration.

**RESULTS**

**Direct K⁺ Determinations.** Figure 1 shows data from an experiment designed to estimate initial rates of K⁺ influx pumping as related to energy derived from respiration. Table I summarizes data from five such experiments.

The medium contained 40 mm potassium acetate and 120 mm sucrose. Sucrose provides osmotic support and as a non-penetrant minimizes passive swelling and maximizes the active component dependent on influx pumping. No valinomycin or other ionophorous agent is needed for influx pumping; hence, this is "spontaneous" active accumulation (6). On cessation of respiration due to NADH exhaustion there is shrinkage as accumulated salt diffuses back out to passive equilibrium. By using υ-C-sucrose and THO, calculations can be made of the sucrose inaccessible, or osmotically responsive, space which is generally equaled with matrix space. Potassium concentrations in this space were determined during passive swelling, active swelling, and passive shrinkage by assuming that the sucrose accessible space was in equilibrium with the potassium in the external medium. Proton movements were determined from the pH recording and the acid titer. The H⁺ consumed during NADH oxidation (NADH + H⁺ + 1/2 O₂ → NAD⁺ + H₂O) was subtracted from this amount to give an estimate of the net H⁺ efflux during the reaction. NADH oxidation rates were determined from O₂ consumption.

During the early phase of passive swelling there is an entry of K⁺ and a rapid exit of H⁺. Probably a portion of the K⁺ entry is in exchange for H⁺, especially immediately following addition of the mitochondria to the medium (13). However, there is also a small degree of passive swelling even in this sucrose-supported medium indicating a passive K-aceate influx. In the experiment of Figure 1, the sucrose inaccessible space actually declines slightly but this is not typical. In three of the five experiments, there was a small increase in sucrose inaccessible space and on the average no significant change. The volume increase due to passive salt entry indicated by the absorbance trace is too small to be accurately reflected in the sucrose inaccessible space measurements.
Introduction of NADH initiated a constant rate of respiration paralleled by a constant rate of alkalinization of the medium (Fig. 1). NADH was oxidized at 260 nmoles/min·mg protein as calculated from oxygen consumption. Corn mitochondria, like other plant mitochondria, oxidize exogenous NADH with P/O ratios between 1 and 2, and acceptorless respiration is insensitive to rotenone and amytal (12, 34). Assuming a maximum of 2 equivalents of high energy bond formed per NADH oxidized, the rate of I → X formation was 520 nmoles/min·mg protein.

After correction for H⁺ consumed in NADH oxidation on the basis of H⁺/O = 1, the rate of H⁺ ejection was 22 nmoles/min·mg protein (Fig. 1). Net proton ejection was thus trivial compared to the rate of I → X formation. The calculated H⁺ ejection was highly variable and, although linearly related to respiration, showed no evident stoichiometry. The highest rate of 150 nmoles H⁺/min·mg protein (Table I) was obtained with mitochondria which appeared to be somewhat uncoupled endogenously. Packer and Utsumi (26) equate high rates of H⁺ ejection with membrane damage.

By fitting tangents to the K⁺ content curves estimates can be made of initial rates of K⁺ influx. In Figure 1 the influx rate of K⁺ into total space was 492 nmoles/min·mg protein, and that into the sucrose inaccessible space was 472 nmoles/min·mg protein. Within error of measurement these figures are essentially the same, and it can be reasonably deduced that active K⁺ uptake is into the matrix.

Uptake of K⁺ shows steady state kinetics, and parallels the light transmission curve. (In Figure 1 the K⁺ content curves are somewhat offset due to difficulties in exact timing imposed by centrifuging. Electrode determinations of K⁺ in Figures 2 and 3 show the parallelism.) Steady state equilibrium was not obtained in these experiments before exhaustion of NADH, and thus the initial K⁺ efflux rates on cessation of respiration are not as high as initial influx rates. In Figure 1 the initial rate of passive back diffusion from the sucrose inaccessible space was 106 nmoles/min·mg protein. It appears that there must be a concentration gradient of potassium acetate built up such that back diffusion through the membrane becomes very rapid, eventually offsetting the respiration-linked influx pump.

Calculations on the basis of total water and K⁺ uptake for the 5 experiments show a mean value of 115 mm K⁺ entering the mitochondria. Since an equivalent of acetate enters with the K⁺ (35), the entering solution would be 230 millimolar. Wilson et al. (35) previously found the uptake of potassium acetate to be accompanied by an osmotic equivalent of water. However, the same calculations for the sucrose

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**Fig. 1.** Fluxes during active swelling in potassium acetate. The reaction medium contained 40 mM tris potassium acetate, 120 mM sucrose, 0.5 mM TES-tris, pH 7.3, and 1 mg bovine serum albumin/ml. Volume = 3.3 ml, temperature = 25°C, mitochondrial protein = 6.32 mg. See “Materials and Methods” for details.

**Table I. Initial Flux Rates during Respiration-driven Acetate Swelling**

Experimental conditions are described in “Materials and Methods” and Figure 1, which illustrates experiment 4. NADH oxidation rates were determined from O₂ consumption rates.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Temp</th>
<th>Respiration Rate (nmoles/mg)</th>
<th>K⁺ Uptake Rate (nmoles/mg)</th>
<th>K⁺ Uptake ~H⁺ Ejection Rate (nmoles/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N°</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>260</td>
<td>520</td>
<td>350</td>
</tr>
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<td>2</td>
<td>26</td>
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<td>25</td>
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<td>472</td>
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<td>5</td>
<td>25</td>
<td>216</td>
<td>432</td>
<td>420</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Potassium flux during active swelling determined with the K⁺ electrode. The reaction medium contained 40 mM tris potassium acetate, 1 mM KCl, 120 mM sucrose, 0.5 mM TES-tris, pH 7.2, and 1 mg bovine serum albumin/ml. Volume = 3.4 ml, temperature = 24.6°C, mitochondrial protein = 5.04 mg. Valinomycin added in b = 0.35 μg.
inaccessible space are quite a bit higher—164 mM K⁺ in the entering solution. There is indication in this discrepancy that some sucrose may be penetrating into the matrix space, giving spuriously low calculated values for the water content of this space. Another observation suggestive of sucrose penetration is the failure to obtain complete reversal of the active swelling on cessation of respiration (Fig. 1). Extensive swelling may alter the sucrose permeability of the inner membrane. In a recent review. Tedeschi (32) discusses comparable problems of very high concentration of K⁺ calculated for sucrose-inaccessible space in animal mitochondria and suggests the two-space model may be inadequate.

By using the initial rates of K⁺ entry and NADH oxidation, the K⁺/NADH ratios were calculated for the 5 experiments (Table I). The values approach unity, indicating that 1 molecule of potassium acetate is transported inwards for each coupling equivalent.

Cation Electrode Experiments. The K⁺ electrode was used to obtain more direct and sensitive determinations of initial influx rates. A substitution was made of 40 mM tris acetate + 1 mM KCl for the 40 mM K⁺ of the preceding experiments, thus bringing the K⁺ concentration into the sensitivity range of electrode. However, little change in K⁺ activity was found on introduction of NADH, although the mitochondria actively swollen (Fig. 2a). There is a ready explanation for this. Wilson et al. (35) found that the cation used with acetate had little effect on active swelling, tris being about as effective as K⁺. Proportional uptake of tris and K⁺ accompanied by osmotic adjustment would not produce a change in K⁺ activity. However, there was a brief burst of K⁺ uptake immediately after adding NADH which might represent cation exchange.

In order to increase selectively K⁺ influx over tris, valinomycin was added to the medium (Fig. 2b). Valinomycin had the desired effect, and an initial K⁺ influx rate of 500 nmoles/invert mg protein was obtained. Since NADH oxidation rates were linear (Fig. 1), oxidation rates could be calculated from the time required to exhaust the NADH. In Figure 2b the NADH was consumed at 310 nmoles/min·mg protein corresponding to 620 nmoles/minute·mg protein, and giving a K⁺/NADH ratio of 0.81. This value is in the range found by the centrifugal filtration and K⁺ assay technique (Table I).

It should be noted in Figure 2b that after the initial rapid flush of K⁺ uptake there was a backflow of K⁺ prior to exhaustion of NADH. This indicates K⁺-tris⁺ exchange since the mitochondria remain swollen until the NADH is exhausted.

Corn mitochondria show little passive swelling in magnesium acetate compared to potassium acetate, although active swelling occurs in both (35). One might expect, therefore, that from a mixture of the cations, K⁺ would preferentially accompany acetate. Thus Mg²⁺ was substituted for tris⁺ in an experiment like that of Figure 2. As shown in Figure 3, it was now possible to observe a definite decline in K⁺ activity of the medium with active swelling, and this was accentuated with valinomycin. Unlike the experiments in tris acetate, however, no backleak of K⁺ into the medium was seen until NADH was exhausted, indicating little or no loss of K⁺ by exchange with Mg²⁺. Slower swelling and respiration rates in the presence of Mg²⁺ are ascribed by Brierley et al. (6) to decreased permeability to K⁺.

The value obtained from Figure 3b for initial K⁺ influx was 260 nmoles K⁺/min·mg protein. NADH oxidation was 368 nmoles/min·mg protein, giving a ratio of 0.36 K⁺/NADH. This low value suggests Mg²⁺ as well as K⁺ accompanied the acetate.

Stoichiometry of Active Contraction in Potassium Chloride. Figure 4 gives data from an experiment like that shown in Figure 1, but with substitution of 100 mM KCl for sucrose and potassium acetate, thus permitting passive swelling followed by active KCl extrusion (35). Passive swelling was associated with a slow rate of H⁺ loss to the medium. On introduction of NADH there was a constant rate of oxygen consumption and H⁺ ejection. However, the K⁺ which had been passively absorbed was now pumped out of the inner mitochondrial space. When the NADH was exhausted, the mitochondria again swelled passively, but at a greater rate than initially.

The initial rate of efflux pumping was graphically estimated...
to be 320 nmoles K'/min·mg protein and 27 nmoles H+/min·mg protein. NADH oxidation calculated from O2 consumption was 174 nmoles/min·mg protein, corresponding to 348 nmoles ~'/min·mg protein, and giving a K'/~/ ratio of 0.92. If K' efflux rate and H+ ejection rate are summed, a total cation efflux rate of 347 nmoles/min·mg protein is obtained with a cation ejected ~/ ratio of 1.0.

Chloride would be expected to be the major anion accompanying the ejected K' (1, 2, 4). Analyses made of five sets of swollen and contracted mitochondria for K' and Cl- contents (Table II) show a small excess of K' over Cl-, similar to that shown in liver mitochondria (2). The differential between K' and Cl- ejected during respiration might indicate a loss of endogenous anions.

Effect of Valinomycin on Contraction and Respiratory Control. A few experiments were conducted to determine the effect of valinomycin on mitochondrial contraction and respiratory control ratios. As shown in Figure 5, valinomycin at 10 ng/ml greatly accelerated passive swelling in 0.1 M KCl (note absorbance at 1 min) but did not interfere with the efflux pump. An uncoupling effect of valinomycin on respiration was found and is shown in Figure 5 in the shorter time required to exhaust the NADH, permitting reswelling. No permeant anion was required for valinomycin uncoupling.

Uncoupling with valinomycin was further examined in a medium containing 0.2 M sucrose, 0.5 mM TES-tris, pH 7.3, 1 mg bovine serum albumin/ml, and 2 mM tris phosphate. Without valinomycin a mean respiratory control ratio with NADH of 2.8 was obtained. With addition of 0.023 µg valinomycin/mg mitochondrial protein, the respiratory control ratio dropped to 2.0. When 1 mM KCl was also added the respiratory control ratios dropped to 1.25. In rat liver mitochondria Mitchell (25) has reported that respiratory control is maintained with valinomycin, and Rottenberg (30) found valinomycin plus 10 mM K+ to permit good P/O ratios.

H+ Ejection versus External K+ Concentration. It was observed in both influx and efflux pumping that the calculated H+ ejection was linear and continuous throughout respiration. On the other hand, net K+ flux in either direction showed steady state kinetics, with rapid initial rate falling as a countercflow of salt was established. Only K+ was involved in this back diffusion—the small amount of H+ that was ejected did not reenter the mitochondria (Fig. 4). Impermeability to H+ is a tenet of Mitchell's chemiosmotic hypothesis (23, 24).

Since H+ ejected constituted such a small portion of the total cation efflux, an experiment was designed to determine if H+ ejection could be increased by lowering the KCl concentration of the medium. Figure 6 shows that the opposite result was obtained. When sucrose substituted for KCl, almost no H+ was ejected. With added KCl, H+ ejection increased and remained constant over the range of 20 to 100 mM KCl. Respiration rates on the other hand increased steadily over this range. Höfer and Pressman (18) found increased respiration and phosphorylation in liver mitochondria suspended in a sucrose medium upon addition of K+ and valinomycin.

DISCUSSION
Isolated plant mitochondria are characteristically more permeable than animal mitochondria as evidenced by rapid passive swelling in salt solutions. Endogenous respiration is very low, possibly due to loss of substrates during isolation. NADH is rapidly oxidized and is a preferred substrate, since it does not produce an oxidizable or inhibitory product, nor is it a "permeant" anion. These characteristics provide a relatively simple system in which passive and active fluxes of salt can be observed at neutral pH and in which respiration can be stopped without the use of inhibitor by simply limiting the quantity of NADH added. Ionophorous antibiotics are not needed.

Under these conditions the initial rates of both influx pumping of K acetate and efflux pumping of KCl were found to approach 1 molecule of salt per hypothetical high energy intermediate. The closest comparison with animal mitochondria

**Table II. K+ and Cl- Contents of Mitochondria**

<table>
<thead>
<tr>
<th></th>
<th>K+</th>
<th>Cl-</th>
<th>A-</th>
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<tbody>
<tr>
<td></td>
<td>µmoles/mg protein</td>
<td></td>
<td></td>
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<tr>
<td>Swollen</td>
<td></td>
<td>1.32</td>
<td>0.23</td>
</tr>
<tr>
<td>Contracted</td>
<td>1.33</td>
<td>1.13</td>
<td>0.20</td>
</tr>
<tr>
<td>Extruded</td>
<td>0.22</td>
<td>0.19</td>
<td>0.03</td>
</tr>
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</table>
lies in the work of Brierley et al. (6) who used beef heart mitochondria without valinomycin and obtained 1.1 to 1.4 K+/~ in active K+ uptake from K acetate. Both sets of values are considerably lower than those from rat liver mitochondria in the presence of valinomycin (see Introduction).

Use of valinomycin with corn mitochondria poses problems, since the ionophore appears to have uncoupling properties, releasing acceptorless respiration. Where influx pumping of K acetate is followed (Fig. 2), the uncoupling might be ascribed to facilitating K+ penetration, giving greater rates of potassium acetate uptake and increasing the expenditure of respiratory energy. However, with efflux pumping of KCl (Fig. 5) the action of valinomycin is much like that described for the classical uncoupler, 2,4-dinitrophenol (14). That is, passive permeation of KCl is increased, but without drastic change in the active efflux pumping which produces the shrinkage. Efficiency of efflux pumping, however, is lowered due to the stimulation of respiration. As reported above, valinomycin also lowers respiratory control. Other aspects of valinomycin uncoupling are reported in another paper (16), where it is suggested that facilitating the transport of H+ with uncouplers or K+ with valinomycin may promote hydrolysis of a primary cova lent bond (1 ~ X) formed at coupling sites, collapsing the membrane potential and releasing respiration.

At this time it is difficult to attribute any specific significance to a K+/~ ratio of about unity. The ratio as calculated assumes perfect coupling between electron transfer at coupling sites and ion transport. However, P/O, or ADP/O ratios in corn mitochondria with NADH as substrate are reported to be 1.2 to 1.8 (12, 16, 34). If ion transport has the same efficiency of coupling, the K+/~ ratios could be increased up to 50%. The question here is whether or not the coupling to phosphorylation and to potassium acetate transport should be expected to have the same efficiency. At present this question cannot be answered.

The K+/~ ratio takes on more significance in efflux pumping of KCl. Brierley’s (4) explanation here is that a proton motive force set up by respiration is collapsed by exchanging external H+ for internal K+, accompanied by exit of the chloride ion to maintain electroneutrality. Brierley thus supports Mitchell’s (23, 24) chemiosmotic hypothesis. Assuming that 2 H+ are extruded at each respiratory “loop” (23, 24), and that exogenous NAD is oxidized through two loops, we should have observed K+/~ = 2. Since the observed ratio is half this and since K+ appears essential to proton ejection and respiration, the mechanism of coupling respiration to efflux transport may not be through respiratory loops. The observations fit better with the hypothesis of our laboratory that the coupling act involves the polarized extrusion of H+ (or K+) and OH- during the dehydration essential to production of ~ X (15, 16, 35). Here a ratio of K+/~ = 1 is expected, and a dependence on K+ allowed. Failure of valinomycin to block contraction can be explained by failure of the ionophore to interfere with ~ X formation and K+ extrusion.

LITERATURE CITED