

On the Mechanism of Regulation of the Mitochondrial K^+/H^+ Exchanger*

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1. Rat liver mitochondria undergo a spontaneous potassium efflux in hypotonic sucrose. This reaction is electroneutral, and therefore cannot reflect electrophoretic K^+ efflux through damaged membranes. Swelling-induced K^+ loss does not require respiration, and it is associated with proton uptake. Swelling is therefore considered to unmask an endogenous K/H exchanger.

2. The extent of swelling-induced K^+ loss increases with the extent of swelling; however, the gradients driving K/H exchange decrease with increased swelling. Therefore, the mitochondrial K/H exchanger must be regulated by a mechanism not dependent on gradients of K^+ or H^+ .

3. In 75 mM sucrose at $0^\circ C$, endogenous K^+ declines from 130 to 70 $\mu mol/g$ in 15 min, after which no further loss occurs. When mitochondria are contracted by rapid additions of hypertonic sucrose, K^+ efflux ceases abruptly. Restoration of K/H exchange in K^+ -depleted preparations is readily achieved by a period of respiration at $25^\circ C$ in 0 mM K^+ . During swelling, K^+ is lost from an osmotically active pool of K^+ , and this loss leads to a secondary matrix contraction. It is proposed that the exchanger is regulated by a matrix "carrier brake" mechanism which is reversibly released by swelling and whose role *in vivo* is to provide volume homeostasis.

4. Swelling-induced K^+ loss is strongly affected by the affinities of matrix anions for M^{2+} . It is proposed that matrix Mg^{2+} ions bind reversibly to the K/H exchanger, preventing K^+ efflux and acting as a carrier brake. When free Mg^{2+} concentration is reduced by dilution (swelling) or complexation with anions, the exchanger is released from inhibition and electroneutral K^+ efflux ensues. The carrier brake hypothesis is consistent with the chemiosmotic theory, and it provides a sensitive homeostatic mechanism for regulation of potassium and volume without excessive futile cycling of K^+ .

Attempts to integrate mitochondrial ion transport with the chemiosmotic theory of energy coupling have been increasingly successful during the past decade. It has nevertheless proved very difficult to accommodate observations on potassium transport to the elegant uniport-antiport scheme proposed by Mitchell (1). This discrepancy between theory and experiment has led to a wide variety of proposals: K^+ uniport and K/H antiport are linked in some manner, both to each other and to the energy state (2-4); there is a potassium pump

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(5-12); K/H exchange is not electroneutral (13); the chemiosmotic theory is in need of modification (14) or flatly incorrect (10, 11, 15). Indeed, doubts have been raised that mitochondria even possess electroneutral K/H exchange activity (11, 13, 16). Thus, the mechanisms of K^+ transport in mitochondria remain an unresolved and controversial subject (see Lehninger, *et al.* (17) and Brierley (4, 18) for reviews).

Sometimes it is possible to gain insight into such mechanisms by consideration of the physiological constraints imposed on the system by its environment. Chemiosmotic mitochondria respiring *in vivo* are infinite sinks for electrophoretic K^+ uptake, and swelling and lysis would ensue if there were no compensatory mechanism for K^+ ejection. This is due to the high membrane potential, to the high concentration of K^+ in the cytosol, to the high permeability of the inner membrane to water, and to anion transporters which are ΔpH -sensitive. Even a modest potassium traffic would require that the K^+ uniport and antiport mechanisms remain in balance in order to maintain mitochondrial volume homeostasis *in vivo*. We may safely conclude that a regulatory mechanism for one or both pathways is a physiological necessity for chemiosmotic mitochondria.

It also seems evident that the K/H antiporter, if it exists, must be regulated by a mechanism not involving ΔpH . This conclusion is drawn from an experiment frequently performed by researchers who study these organelles. When mitochondria are isolated and purified in a potassium-free medium, they have an alkaline matrix ($\Delta pH > 1.0$), and they retain high K^+ (130 $\mu mol/g$) for long periods (19). Thus, K/H exchange does not occur despite gradients of both K^+ and H^+ which overwhelmingly favor such a reaction. The simplest interpretation of this finding is that the hypothetical antiporter must be regulated, not by gradients of K^+ or H^+ , but by some other mechanism which I have called a "carrier brake"¹ (20, 21).

I have previously shown in a brief report (22) that hypotonic swelling induces K/H exchange in rat liver mitochondria. Swelling-induced K^+ loss is readily observed in simple media at $0^\circ C$, a temperature at which electrophoretic cation movements are minimized. The present report extends the previous studies and deals primarily with factors affecting the magnitude, rather than the kinetics, of K^+ efflux induced by swelling. This choice of protocol was motivated by the desire for simplicity in the interpretation of the experimental results. The conclusions of this investigation are that hypotonic swelling unmasks the physiological K/H exchanger; that control of K/H exchange is mediated by a matrix solute acting as a carrier brake; and that the carrier brake involves Mg^{2+} ions.

¹ "Carrier brake" is a simple descriptive term for a complex, hypothetical control mechanism. This terminology is consistent with the present findings without implying knowledge of the detailed molecular interactions.

The physiological role of this mechanism is to maintain mitochondrial volume homeostasis with minimal energy expenditure. The carrier brake model provides a simple framework for the integration of mitochondrial K^+ transport with the chemiosmotic theory of energy coupling and the general energy economy of the cell.

EXPERIMENTAL PROCEDURES

Materials—Rat liver mitochondria were isolated according to the high yield, differential centrifugation technique of Pedersen *et al.* (23), modified by isosmotic substitution of sucrose for mannitol. Stock suspensions containing 50 mg of protein/ml were stored in 0.25 M sucrose at 0°C prior to use.

Osmotic Swelling Protocol—In a typical swelling study, 0.5-ml aliquots of the stock suspension were added to 5 ml of media containing sucrose buffered at pH 7.0 (at 0°C) with 15 mM TEA TES.² Incubations were carried out with gentle agitation in an ice water bath for 15 min based on the finding that swelling-induced K^+ loss is complete within that time (22). When radioactively labeled sucrose, acetate, and/or water were used, they were added 1 min prior to centrifugation for 5 min at 15,000 × *g* in a Sorvall RC2B refrigerated centrifuge.

Extractions and Assays—The supernatant was decanted, and the tube containing the pellet was swabbed. Aliquots of the supernatant were assayed for osmolality by freezing point depression (Precision Instruments Osmette) and for density (Mettler/Par density meter). The pellet was suspended to homogeneity with H₂O and an aliquot was taken for biuret protein (24). Another pellet aliquot was added to 0.1 M ammonium acetate, pH 4.55, containing 4 mM CsCl and 0.2% LaCl₃. This mixture was heated at 90°C for 3 min, and the resulting clear pellet extract was decanted following cooling and centrifugation. This extraction procedure, based on that of Walser (25), leads to complete recovery of cations and minimal quenching during scintillation counting. The supernatant was extracted in identical media and all samples were assayed for Na⁺, K⁺, Mg²⁺, and Ca²⁺ by atomic absorption spectroscopy and for radioactivity (in Beckman's Redisolv mixture) by liquid scintillation counting. The matrix water content was obtained as previously described (26). Pellet dry weight, corrected for the weight of solutes trapped in the sucrose space, was used as a normalizing factor for ions and water content. Matrix K⁺ content, K_m (micromoles per g), was corrected for K⁺ in the sucrose space; however, this correction was very small (<1%) due to the low external K⁺ in these studies (<0.25 mM at the start of each run).

Kinetic Studies—Ion electrode studies were carried out on 9-ml samples containing 4 mg/ml of mitochondrial protein. The smooth curves plotted in Figs. 1 and 6 represent data calculated from potentials printed at 0.1-min intervals as previously described (45). Photometric studies were carried out using a light probe connected to a Brinkmann photometer. The light probe was immersed in 4.0 ml of media containing 0.2 mg/ml of mitochondrial protein.

RESULTS

The Relative Kinetics of Swelling and K⁺ Efflux in Hypotonic Media—This report will focus primarily on conditions which affect the magnitude, rather than the kinetics, of swelling-induced K⁺ loss. Nevertheless, it is important to establish the disparity between the rate of swelling and the rate of K⁺ loss, since both processes are time-dependent (see Fig. 1). While the half-time for swelling-induced K⁺ loss is about 2.0 min, the rate of swelling is too rapid to measure, being complete within seconds (27). The slow, secondary contraction of the matrix in hypotonic media follows the time course of the K⁺ loss, which strongly suggests that the efflux is from an osmotically active pool of K⁺. The rapidity of swelling in hypotonic media most likely reflects the high surface/volume ratio and the high permeability of the inner membrane to water. For the purpose of this study, we may safely assume that hypotonic swells are virtually instantaneous when measured on the time scale of swelling-induced K⁺ loss.

² The abbreviations used are: TEA, tetraethylammonium ion; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; P_i, inorganic phosphate; mosm, milliosmolar; K_m , matrix K⁺ (micromoles per g); W_m , matrix water content (grams per g).

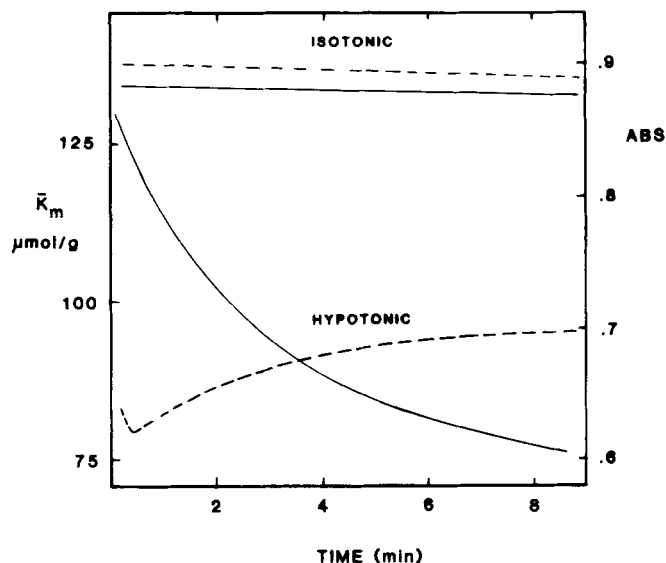


FIG. 1. Comparative kinetics of swelling and swelling-induced K⁺ loss. The smooth curves represent the time course of K_m (micromoles per g) as determined from ion electrode potentials. The dashed curves represent the time course of absorbance readings obtained in identical media. Incubations were carried out at 0°C in 272 mosm media (upper curves, labeled isotonic) or in 75 mosm media (lower curves, labeled hypotonic). External K⁺ concentration was 0.25 mM at the start of all incubations.

The Dependence of Matrix K⁺ on Osmolality—A range of matrix volumes was obtained by varying the osmotic strength of the incubation medium from 70 to 550 mosm. The osmotic strength of 0.25 M sucrose (272 mosm) represents the isosmotic point of reference for swelling and shrinking, since mitochondria are isolated and stored in this medium. The water content (W_m) of mitochondria in 0.25 M sucrose is normally found to be 0.96 g/g in our laboratory.

The relationship between matrix K⁺ and inverse osmolality ($1/\Phi$) following 15 min of incubation of 0°C is shown in Fig. 2 (crosses). In swollen mitochondria, the extent of K⁺ loss is monotonically dependent on $1/\Phi$ and therefore on the extent of swelling. The extent of K⁺ loss in swollen mitochondria is highly significant when compared to negligible losses in controls: following 0-, 15-, and 60-min incubations in isosmotic sucrose, matrix K⁺ was found to be 131 ± 2 , 130 ± 2 , and 129 ± 2 $\mu\text{mol/g}$, respectively, confirming our previous finding that K⁺ changes very slowly at 0°C in 0.25 M sucrose (19). The closed circles plotted in Fig. 2 represent values obtained on a preparation which was depleted of K⁺ by swelling, resuspended in 0.25 M sucrose, and subjected to the osmotic swelling series. This experiment demonstrates that once K⁺ loss has been induced at a given matrix volume, no further K⁺ loss occurs upon shrinking and reswelling.

In summary, a transient K⁺ efflux is induced in hypotonic media. The magnitude of swelling-induced K⁺ loss is limited and depends on the magnitude of swelling.

Inhibition of Swelling-induced K⁺ Loss by Acetate and Malate—The presence of 20 mM TEA⁺ acetate or malate in the hypotonic media completely inhibited K⁺ loss. Preincubation in 100 mM K⁺ malate, followed by sucrose washes, also resulted in inhibition of swelling-induced K⁺ loss (Fig. 2, open circles).

The Reversibility of Swelling-induced K⁺ Loss—Mitochondria were first depleted of K⁺ by swelling, then incubated in 100 mM K⁺ and substrate for 30 min at 25°C, followed by a cold sucrose wash. Potassium was found to be 180 to 200 $\mu\text{mol/g}$ in both depleted and fresh mitochondria, despite

different preincubation values of 70 $\mu\text{mol/g}$ and 130 $\mu\text{mol/g}$, respectively.

Restoration of matrix K^+ is not sufficient to establish the reversibility of the swelling-induced K^+ loss reaction. Accordingly, the pretreated mitochondria described above were next subjected to an osmotic swelling series. The results are shown in Fig. 3. Although matrix K^+ begins higher (195 $\mu\text{mol/g}$) and ends lower (51 $\mu\text{mol/g}$), it is evident that swelling-induced K^+ loss has the same characteristics as those observed in fresh mitochondria (compare Fig. 2). It is noteworthy that the curves obtained from depleted, preincubated mitochondria are indistinguishable from those obtained from nondepleted, preincubated preparations. As observed with fresh preparations (Fig. 2), acetate and malate (20 mM) completely prevented K^+ loss when present in the hypotonic medium (not shown). A brief (5 min) exposure to 100 mM K^+ malate, followed by a sucrose wash, also resulted in inhibition of swelling-induced K^+ loss (Fig. 3, *open circles*).

The process of swelling-induced K^+ loss is therefore reversible, and the capacity of mitochondria to carry out this process is not lost after swelling. The similar responses of control and preswollen mitochondria suggest that swelling-induced K^+ loss does not reflect differential effects of swelling on a heterogeneous population of mitochondria.

The Effect of Swelling on Other Endogenous Cations and on Matrix pH—Mitochondria do not retain Na^+ during the isolation procedure (28), and the low levels observed (1.5 \pm 1 $\mu\text{mol/g}$) were not affected by swelling. Endogenous Mg^{2+} (38 \pm 1 $\mu\text{mol/g}$) and Ca^{2+} (5 \pm 1 $\mu\text{mol/g}$) were also unaffected by swelling.

The pH gradient was estimated from [^3H]acetate distributions in freshly isolated mitochondria suspended in 0.25 M sucrose buffered at pH 7.0 at 0°C. The observed gradients

correspond to a matrix pH of 8.3 to 8.5. After 15 min in 80 mosm sucrose, followed by centrifugation and resuspension in 0.25 M sucrose, the K^+ loss was 50 $\mu\text{mol/g}$ and the matrix pH was found to be 7.4 to 7.6, an acidification of about 1 pH unit. It was also found that inorganic phosphate declined from 15 $\mu\text{mol/g}$ in fresh mitochondria to 9 $\mu\text{mol/g}$ after swelling.

The Effect of Permeant Non-electrolytes on Swelling-induced K^+ Loss—To establish a direct relationship between swelling and K^+ loss it is necessary to rule out effects of the decrease in water activity. Therefore, isosmotic (constant water activity) swelling studies were carried out in the presence of urea and dimethylsulfoxide whose concentrations were adjusted so that the final tonicity of all solutions was 272 \pm 10 mosm. Incubations were extended to 25 min to assure that equilibrium volume was attained. As expected, equilibrium water content depended solely on the concentration of sucrose, due to the equilibration of the non-electrolyte across the inner membrane. It was found that addition to non-electrolytes had no effect on the magnitude of K^+ loss at a given matrix volume; therefore, matrix swelling, and not the increase in water activity, induced the transient K^+ loss.

The Effect of Swelling on Cytochromes—Extraction of cytochrome *c* from rat liver mitochondria was found by Jacobs and Sanadi (29) to require a preliminary exposure to hypotonic medium (15 mM KCl) followed by washing in 150 mM KCl. In view of the proposal by Margoliash (30) that cytochrome *c* may mediate ion transport, it was considered desirable to measure the effects of hypotonic swelling on cytochrome content. Mitochondria were swollen for 15 min at 0°C both in 75 mM sucrose and in 60 mM TEA TES, then assayed for cytochromes *a*, *b*, *c*1, and *c* by the method of Williams (31). No difference in cytochrome content was found when swollen mitochondria were compared to controls. This lack of

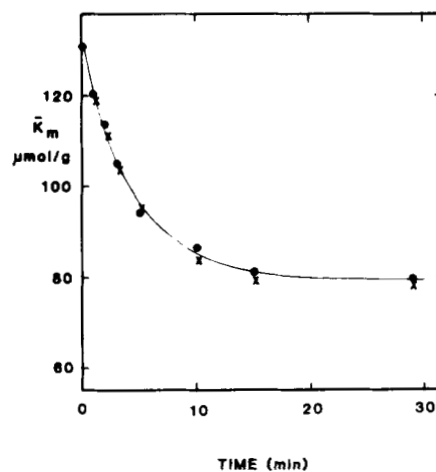
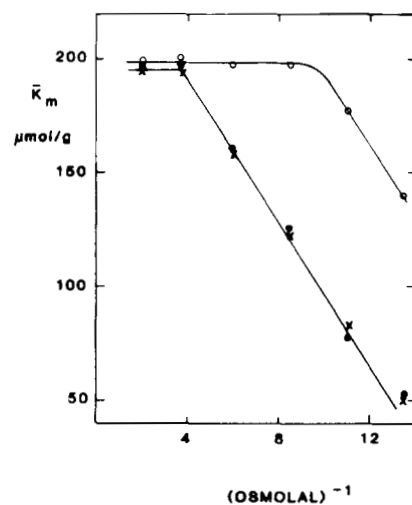
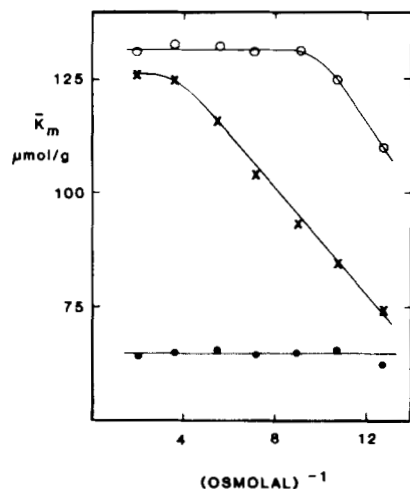


FIG. 2 (*left*). The effects of medium osmolality on mitochondrial K^+ . Following pretreatment, mitochondria were washed and resuspended in 0.25 M sucrose to a final concentration of 50 mg/ml, then incubated for 15 min at 0°C at the indicated osmolality (see "Experimental Procedures"). *Crosses*, no pretreatment; *closed circles*, preincubation for 15 min at 0°C in 80 mosm sucrose; *open circles*, pretreatment with 1.0 $\mu\text{g}/\text{mg}$ of rotenone followed by preincubation for 3 min at 0°C in 100 mM K^+ malate.

FIG. 3 (*center*). Mitochondrial K^+ following a depletion-repletion-depletion cycle. *Crosses*, fresh mitochondria were incubated with K^+ and substrate (K^+ repletion medium) at 25°C for 15 min, then washed and resuspended in cold, 0.25 M sucrose, then subjected to the osmotic series as described under "Experimental Procedures". *Closed circles*, these preparations were treated identically with those described above except that they were first depleted of K^+ by swelling in 75 mosm media for 15 min at 0°C before being

added to the K^+ repletion media. *Open circles*, mitochondria were depleted and repleted as in the previous group, then treated with rotenone (1 $\mu\text{g}/\text{mg}$) and incubated in 100 mM K^+ malate for 5 min at 0°C prior to the cold sucrose wash and osmotic series. The K^+ repletion medium contained sucrose, K^+ salts of 10 mM 2-oxoglutarate, mM P_i , 5 mM MgEDTA, 0.2 mM ADP, 3 mg of protein/ml, at 270 mosm. K^+ was adjusted to 100 mM with KCl. Note that the ordinate scale differs from that of Fig. 2.

FIG. 4 (*right*). The effect of osmotic reversal on swelling-induced K^+ loss. Aliquots of stock mitochondria were added to 4 ml of medium with a final tonicity of 85 mosm. At the times indicated on the abscissa, 1 ml of 1.0 M sucrose was added with rapid mixing. Data were obtained from incubations centrifuged immediately after osmotic reversal (*closed circles*) or 15 min after osmotic reversal (*crosses*).

effect in low ionic strength media is consistent with the reported requirements for extraction of cytochrome *c* (29).

It has been shown by Matlib and O'Brien (32) that 150 mM KCl is sufficient to mobilize cytochrome *c* into the intermembrane space and that the hypotonic treatment serves to release it by rupturing the outer membrane. The low ionic strength of the present experiments is unfavorable for such mobilization; however, I have not tested this possibility.

The Effect of Acute Osmotic Reversal on Swelling-induced K^+ Loss—Is swelling-induced K^+ loss caused by matrix swelling or by an acute stress associated with the rapid volume change? To distinguish between these alternatives, the sensitivity of K^+ loss to brief swelling exposures was measured by osmotic reversal. In this procedure, mitochondria were swollen in 75 mM sucrose, then rapidly contracted by the addition of 1.0 M sucrose at different time intervals. The data from one such osmotic reversal is presented in Fig. 4. This experiment demonstrates that a process of K^+ efflux is initiated by swelling and that this efflux is arrested immediately and permanently when the matrix is restored to its starting volume. Remarkably, osmotic reversal yields a kinetic curve with a time constant (2½ min) very similar to that obtained from ion electrode studies (see Fig. 1).

It is concluded that swelling-induced K^+ loss does not result from stresses associated with acute swelling or shrinking, nor does it reflect the quantum ejection of a volume-dependent amount of K^+ . Rather, swelling-induced K^+ loss is a continuous process, requiring swelling for both its initiation and its continuation.

The Effects of Valinomycin and Rotenone on Swelling-induced K^+ Loss—These agents were used to evaluate the electrical properties of swelling-induced K^+ loss (see Figs. 5 and 6). If K^+ were simply diffusing down its electrochemical gradient through a leaky membrane, then valinomycin should accelerate swelling-induced K^+ loss. In fact, valinomycin inhibited K^+ loss when added before swelling (Fig. 5) and reversed swelling-induced K^+ loss when added after most of the K^+ was lost (Fig. 6). These effects of valinomycin were abolished by rotenone, which blocks endogenous respiration. Additional experiments were carried out in conjunction with those reported in Figs. 5 and 6 which support the conclusion that swelling unmasks an electroneutral K/H exchanger: (i) stoichiometric (1:1) proton ejection accompanied K^+ uptake when the latter was induced by valinomycin after efflux had occurred (*dashed curve* of Fig. 6). These movements of K^+

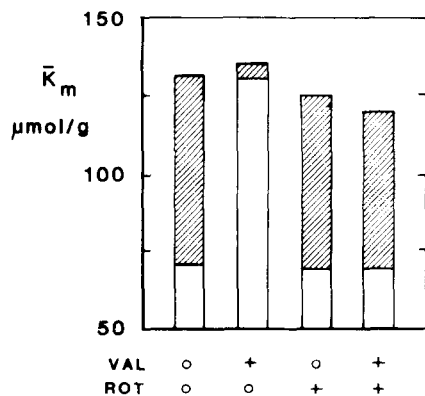


FIG. 5. Effects of valinomycin and rotenone on mitochondrial K^+ . + denotes the presence and o denotes the absence of rotenone (1 μ g/mg) or valinomycin (0.1 μ g/mg) added to the stock mitochondria prior to incubation at 0°C. External K^+ was 0.18 mM at the start of each incubation. The height of the bars represents matrix K^+ (micromoles per g) found after 15 min incubation of 272 mosM (*upper bars*) or 75 mosM (*lower bars*) media. The *shaded portions* represent the swelling-induced K^+ loss under these conditions.

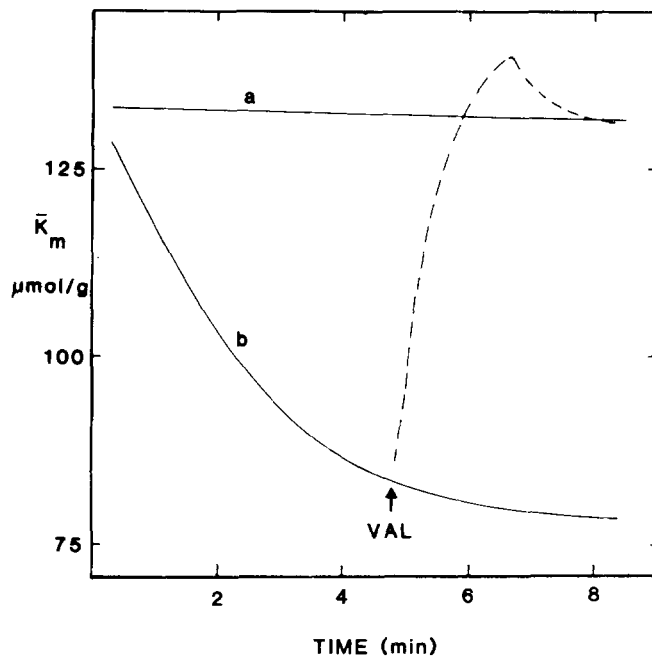


FIG. 6. Effects of valinomycin and rotenone on the kinetics of K^+ transport. Mitochondria were pretreated with 0.1 μ g/mg of valinomycin (*Curve a*) or untreated (*Curve b*), then added to identical media containing 0.18 mM K^+ , 80 mosM, 0°C. The *dashed line* represents the result when the same dose of valinomycin was added to the untreated mitochondria at the time denoted by the arrow (VAL).

and H^+ required energy derived from endogenous substrates, since they were blocked by rotenone. (ii) The uptake of TEA^+ , obtained from [^{14}C]TEA incubations, was found to be less than 0.2 μ mol/(g·min) at 0°C in the presence or absence of rotenone. Passive electrophoretic permeabilities of TEA^+ and H^+ are therefore very low under the conditions of these experiments. (iii) ^{42}K was found to exchange very slowly at 0°C in the absence of valinomycin. Equilibration of ^{42}K was complete and too rapid to measure at 0°C in the presence of valinomycin, with or without rotenone. Because of this high induced permeability, K^+ can be considered to be close to electrochemical equilibrium in the presence of valinomycin. To minimize the energy drain, external K^+ was adjusted to 0.18 mM, a value at which valinomycin caused little or no K^+ movement. Taking internal K^+ to be 150 mM, the membrane potential was about 158 mV in these preparations.

Electrophoretic K^+ movements require a flux of counterions, since there can be no sensible net ionic current across the membrane. Because of the low diffusive permeability of the membrane at 0°C, the only source of counter-ions is the electrogenic proton ejection of respiration. Electroneutral K/H exchange cannot overcome the high electrophoretic permeability induced by valinomycin, so this agent prevents or reverses swelling-induced K^+ loss when respiration provides a counter-current of protons. When respiration is inhibited, no other ionic pathways are available to balance the K^+ current; net electrophoretic flux is prohibited; and valinomycin has no effect on the electroneutral K^+ efflux. The experimental results reported in this section appear to require that swelling-induced K^+ loss is passive and electrically silent. Swelling-induced K^+ loss must therefore reflect the activity of an endogenous, obligatorily coupled K^+ transporter located within the inner membrane.

The Anion Dependence of Swelling-induced K^+ Loss—Further investigation of the effects of anions on swelling-induced K^+ loss were undertaken since these effects appeared

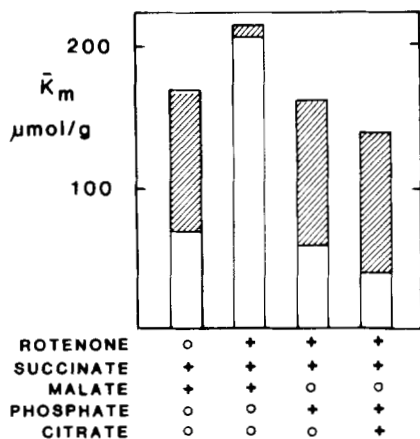


FIG. 7. Effects of anions on swelling-induced K^+ loss. + denotes the presence and o denotes the absence of rotenone (1 $\mu\text{g}/\text{mg}$), succinate (10 mM), malate (5 mM), phosphate (5 mM), or citrate (5 mM). Mitochondria were first depleted of K^+ to 65 $\mu\text{mol}/\text{g}$ by swelling for 12 min at 0°C in 80 mosM sucrose. They were then incubated at 5 mg/ml for 5 min at 25°C in media containing the anions indicated in the figure plus 80 mM K^+ , 15 mM TES buffer, pH 7.2, and sufficient sucrose to maintain isotonicity. The height of the bars represents matrix K^+ (micromoles per g) at the end of this incubation. Next, the samples were washed and resuspended in cold sucrose, then added to 80 mosM sucrose. Following incubations at 0°C for 12 min, pellets were centrifuged and analyzed. The lower bar represents matrix K^+ found after this second exposure to hypotonic media, and the shaded portion represents the extent of the swelling-induced K^+ loss.

to be related to the control mechanism for K/H exchange. To isolate the effects of added anions from those which may result from metabolic intermediates, mitochondria utilizing succinate as a source of energy were preincubated in K^+ at 25°C, then washed and subjected to hypotonic media. Fig. 7 summarizes the effects of anions on the K^+ repletion-depletion cycle. The effect of rotenone (bar 2 versus bar 1) suggests that citric acid cycle intermediates play a role in regulating K^+ efflux. This explanation is consistent with the finding that swelling-induced K^+ loss was stimulated after respiration in 2-oxoglutarate (Fig. 3). In rotenone-treated preparations, substitution of P_i for malate fully restored swelling-induced K^+ loss (bar 3 versus bar 2). P_i plus citrate resulted in swelling-induced K^+ loss greater than that observed in fresh mitochondria (bar 4). These effects, together with acetate inhibition of swelling-induced K^+ loss, are considered to result from the physicochemical properties of matrix anions. Anions which stimulated swelling-induced K^+ loss (citrate and phosphate) have high affinities for divalent cations, while those which inhibited swelling-induced K^+ loss (succinate, malate, acetate) have low affinities for divalent cations.

DISCUSSION

Potassium loss in hypotonic sucrose was first reported in 1966 by Tarr and Gamble (33) without discussion. This phenomenon is not too surprising in view of the many possible changes which may be induced in the membrane by swelling. One explanation which can be excluded by the present study is that swelling-induced K^+ loss reflects membrane leakiness. Increased diffusive permeability to K^+ secondary to membrane "stretching" or damage would be associated with electrophoretic K^+ movements. The experiments reported in Figs. 5 and 6 demonstrate conclusively that swelling-induced K^+ loss is electroneutral. The further findings that swelling-induced K^+ loss is associated with matrix acidification and does not require respiration strongly support the conclusion that swelling unmasks a passive endogenous K/H exchanger by releasing it from inhibition (22).

This conclusion helps to resolve a fundamental question raised by observations that ^{42}K exchange is respiration-dependent (34), stimulated by phosphate (4, 16) and inhibited by phosphorylation (4). These effects appear to require that uniport and antiport activities vary in parallel, which led Brierley (18) to propose that there is a regulated interplay between these two pathways. This raises the question whether the linkage between uniport and antiport is so intimate as to prevent their independent study. Perhaps the most notable success of the present work is the clear demonstration that K/H antiport can be dissected from K^+ uniport under simple experimental conditions. This finding, does not, however, rule out the existence of common pathways for K^+ uniport and antiport or common elements in the control of these pathways under physiological conditions.

Swelling reduces the gradients favoring K/H exchange; nevertheless, K^+ efflux increases with increasing matrix volume (Figs. 2 and 3). These observations support the hypothesis that the mitochondrial K/H exchanger is modulated by a carrier brake mechanism (20–22) and not by gradients of K^+ or H^+ . This reflects physiological constraints: (i) because of the isosmotic co-transport of water (19, 35), the potassium gradient can vary little *in vivo*, even during massive uptake of K^+ salts (20); (ii) because of the co-transport of anions linked to the phosphoric acid uniporter (36, 37), ΔpH can vary little in response to massive swelling and K^+ uptake (20). These teleological considerations beg for an indirect, rather than gradient-mediated, control of the K/H exchanger, and the present data support the existence of such a mechanism. The proposal that the physiological role of the carrier brake is to provide volume homeostasis (22) is also reflected in swelling-induced K^+ loss: The stimulus (swelling) mimics the environmental hazards faced by mitochondria *in vivo*, and the response (release of the K/H exchanger and K^+ efflux) is in the appropriate direction to correct the volume disturbance (see Fig. 1).

Other features of swelling-induced K^+ loss provide strong clues to the identity of the carrier brake: swelling-induced K^+ loss is limited in duration (Fig. 1) and extent (Fig. 2), suggesting release and re-inhibition of the exchanger. The osmotic reversal experiment (Fig. 4) establishes that K/H exchange is not only triggered by swelling but also requires swelling for its continuation. The restoration of carrier activity by respiration in 100 mM K^+ (Fig. 3) strongly suggests that control of the carrier resides within the mitochondrion and that the control mechanism is modified, but not lost, by swelling. These findings appear to require the existence of a matrix solute which binds reversibly to the carrier, preventing carrier movement within the mitochondrial membrane. As volume increases, the concentration of this solute decreases, leading to dissociation of the carrier brake complex which, in turn, permits K/H exchange on the free carrier. This explanation leads to a further restriction on the nature of the carrier brake solute: The total amount in the matrix must be relatively invariant in order for its concentration to vary inversely with matrix volume.

Mg^{2+} ions fulfill the requirement that the total quantity of carrier brake in the matrix should be relatively invariant, since net transport of Mg^{2+} is very slow compared to that of K^+ , Ca^{2+} , or organic anions (38–40). The pronounced effects of matrix anions on swelling-induced K^+ loss (Figs. 2, 3, and 7) strongly implicate divalent cations as the carrier brake or a component thereof. This contention is also supported by the finding that the divalent cation ionophore A23187 induces electroneutral K^+ efflux (41–46), and that this effect of A23187 is due to release of the endogenous K/H exchanger secondary to divalent cation depletion (45, 46). For these reasons, and

because Ca^{2+} stimulates K/H exchange under certain conditions (21), I have proposed that Mg^{2+} ions act to modulate the K/H exchanger (21), a role for Mg^{2+} which has also been proposed by other workers (13, 41, 47). Mg^{2+} may act alone, or it may form a bridge with an inhibitor protein. The present results merely require that the interaction between the brake and the carrier be rapidly reversible. As a working hypothesis, I propose the mechanism illustrated in Fig. 8, which supports and extends Mitchell's postulate for a uniport-antiport mechanism for K^+ transport in mitochondria (1).

To illustrate, consider the operation of this mechanism during isolation of mitochondria in K^+ free 0.25 M sucrose. Initially, potassium and anions will leave the matrix, and the internal concentration of free Mg^{2+} will rise due to the loss of Mg^{2+} complexing anions. Eventually, the carrier will become fully inhibited, resulting in the retention of a major fraction of endogenous K^+ and anions through subsequent sucrose washes. When these mitochondria are subjected to hypotonic media, the concentration of free matrix Mg^{2+} will fall abruptly, resulting in dissociation of Mg-carrier complexes and K^+ efflux on the exchanger. This will lead to acidification of the matrix, further loss of anions via the anion exchangers, and increases in free Mg^{2+} until the K/H exchanger is re-inhibited and K^+ loss ceases. In this general way, the Mg^{2+} carrier brake mechanism is capable of explaining K^+ retention by freshly isolated mitochondria as well as the self-limited loss of K^+ observed during swelling.

The model presented in Fig. 8 predicts that changes in matrix volume, total Mg^{2+} or concentrations of Mg^{2+} complexing anions will have pronounced effects on the activity of the K/H exchanger. Shifts in the energy level should also affect K/H exchange, due to the different affinities of AMP, ADP, and ATP for Mg^{2+} ions. This effect may play a role in the "orthodox" to "condensed" morphological transition observed when aged mitochondria are permitted to phosphorylate ADP (48). This model may also help to explain pathological alterations of mitochondrial volume *in vivo*.

The Mg^{2+} carrier brake mechanism is finely tuned to regulate volume *in vivo* and this is its most important quality. The essential point is that anions which are capable of undergoing net uptake with K^+ include citrate and other anions which form stable complexes of Mg^{2+} . Small fluctuations in free anion content must necessarily result in fluctuations in free Mg^{2+} , changes in the degree of carrier-braking, and variations in K^+ efflux on the exchanger. In the steady state, the system will be in poise with respect to Mg^{2+} -braking such that K/H exchange precisely balances electrophoretic K^+ influx, and constant volume is maintained. By attuning efflux to influx in this manner, the Mg^{2+} carrier brake model prevents excessive

futile cycling of K^+ and minimizes the energetic cost of doing business in a high potassium environment.

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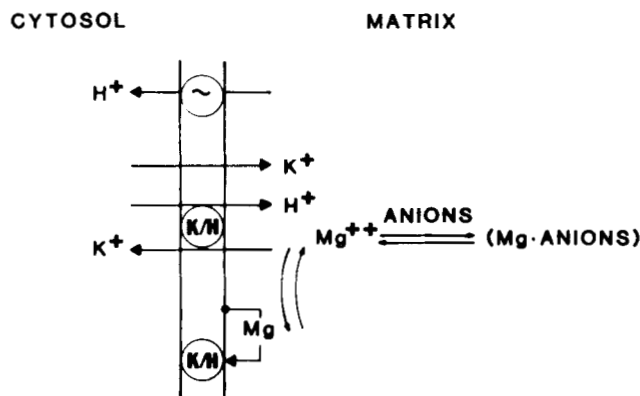


FIG. 8. The Mg^{2+} carrier brake model for regulation of the mitochondrial K/H exchanger. (See text for discussion).

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