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The Mechanism of Mitochondrial Swelling. VIII. Permeability of Mitochondria to Alkali Metal Acetates

George A. Blondin* and David E. Green

Institute for Enzyme Research, University of Wisconsin
Madison, Wisconsin 53706

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Abstract

The capacity of beef heart mitochondria to undergo osmotically induced volume changes in decimolar M^+-acetate or other weak acid anion media is characterized by the following features: (1) mitochondria resist swelling when suspended in potassium or rubidium acetate media in the presence of respiratory inhibitors; (2) mitochondria swell extensively when suspended in ammonium or sodium acetate media in the presence of respiratory inhibitors; and (3) actively respiring mitochondria swell extensively whether suspended in ammonium, sodium, potassium, or rubidium acetate media. These findings have been interpreted to mean that (1) the nonenergized mitochondrial inner membrane is permeable to acetate anions, (2) the nonenergized mitochondrial inner membrane is permeable to ammonium and sodium ions in the presence of acetate or other weak acid anions, (3) the nonenergized mitochondrial inner membrane is relatively impermeable to potassium and rubidium ions in the presence of acetate or other weak acid anions, and (4) energized mitochondria are considerably more permeable to potassium and rubidium (acetate) ions than are non-energized mitochondria. The experiments described in this communication which provide the evidence for these interpretations involve methods which are independent of volume changes. The results confirm the first three of the above interpretations but are inconsistent with the fourth. A general theory for passive ion movements in mitochondria is presented and the results are discussed in terms of the development of an energy dependent ion gradient as the key to energized swelling in potassium or rubidium acetate.

Introduction

The capacity of mitochondria to undergo pseudoenergized† swelling when suspended in decimolar ammonium or alkali metal salt media has been shown by many investigators[1–5] to be, at least in part, a function of the nature of the anion component of the salt. In general, mitochondrial swelling is extremely slow in presence of ammonium or alkali metal salts of strong acid anions such as chloride and bromide. On the other hand, in the presence of weak acid anions‡ such as acetate, propionate, or phosphate, pseudoenergized swelling is relatively rapid when ammonium or sodium is the counter ion while it remains extremely slow when potassium or rubidium is the counter ion. This dichotomy does not exist in the chloride or bromide series, i.e., ammonium, sodium,

* Established Investigator of the American Heart Association.

† Pseudoenergized swelling is operationally equivalent to passive swelling. For a fuller definition of the term "pseudoenergized", the reader is referred to reference 5.

‡ The distinction between strong and weak acid anions is made on the basis of whether or not a given anion possesses a dissociable hydrogen ion in the neutral pH range.

potassium and rubidium salts may all be viewed as incompetent in terms of their ability to induce rapid swelling.

In terms of the permeability of the mitochondrial membrane to cations, it is clear from the above studies that mitochondria are readily permeable to sodium and ammonium ions given acetate as the counter ion. We would like to adopt the convention at this time of describing the permeability of a given cation as a function of its counter ion, e.g., mitochondria are readily permeable to sodium (acetate) and ammonium (acetate). It is not possible to infer with confidence additional permeability properties on the basis of the observed osmotic responses since mitochondria swell extremely slowly when suspended in media composed of ions in all of the remaining combinations described above. To infer that mitochondria are relatively impermeable to sodium (chloride), potassium (chloride), or potassium (acetate), would require affirmation of the assumptions (1) that salt permeability is always the rate-limiting step in the swelling process, and (2) that equilibration of salt is equivalent to the driving force for water influx.

In a previous communication of this series[6] we presented evidence from exchange diffusion and other studies bearing on the permeability properties of the mitochondria relative to alkali metal salts of strong acid anions. The results of this study led us to the conclusion that the mitochondrial inner membrane was relatively permeable to chloride and bromide ions—a property shared by most other synthetic and biological membranes.[7-10] In particular, mitochondria were found to be permeable to sodium (chloride, bromide), potassium (chloride, bromide), and rubidium (chloride, bromide). Since mitochondria resist swelling when suspended in media isoosmolar with respect to these salts, this study clearly demonstrated the inaccuracy of osmotic responses as a measure of mitochondrial permeability.

The present investigation is a continuation of our previous study now extended into the domain of alkali metal acetate salts. While we have explicitly criticized the assumption that a negative osmotic response is a valid criterion for evaluating the impermeability of the mitochondrial membrane to a given ion pair, we are in agreement with others[1-4] in the view that the mitochondrial membrane must be permeable to salts which are capable of inducing extensive passive (pseudoenergized) swelling. Sodium and ammonium acetate represent two such ion pairs. However, mitochondria resist passive swelling in potassium and rubidium acetate.[3,5] Intuitively, we would anticipate that the passive permeability of potassium (acetate) and rubidium (acetate) should be considerably less than that of sodium (acetate) or ammonium (acetate). The present investigation is addressed to a demonstration of this relationship in an unambiguous and quantitative fashion by exchange diffusion studies.

Methods

The preparation of heavy beef heart mitochondria and the measurement of mitochondrial swelling were carried out according to previously described procedures from this laboratory.[5,6,11] The optical density data described in Fig. 2 were recorded with a Cary Model 14 spectrophotometer, whereas those described in Fig. 4 were obtained with a Beckman DK-2 recording spectrophotometer equipped for time drive operation.

The sodium and rubidium ion content of mitochondrial pellets was determined through the use of the gamma emitting isotopes ^{22}sodium and ^{86}rubidium according to

previously described procedures.[6] ^{42}Potassium was used for the determination of the potassium ion content of mitochondrial pellets by the "eyeball" counting technique as previously described.[6] The determination of the acetate ion content of mitochondrial pellets was made according to the following procedure. The mitochondrial pellets ("eyeballs") obtained from an experiment in which mitochondria were incubated with acetate-2-^{14}C were wet weighed, and then one drop of 0·01 M KOH was applied to each pellet. After air drying overnight the pellets were further dried for 3 hours at 60° in a vacuum oven. After dry weight determination, the pellets were solubilized in 0·01 M cetyl pyridinium chloride (3·0 ml) and 0·2 ml aliquots were planchetted for radioassay in a continuous gas flow counter.

Oxygen uptake was measured with a Clark Type-electrode (Beckman Co., Fullerton, California).

Results

Actively respiring mitochondria swell rapidly when suspended in decimolar sodium, potassium, or rubidium acetate.[11] In the presence of respiratory inhibitors mitochondria resist swelling when suspended in decimolar potassium or rubidium acetate, whereas extensive passive swelling persist when they are suspended in sodium acetate media.[5] Other investigators[3] have concluded from these relationships that actively respiring mitochondria are *permeable* to sodium (acetate), potassium (acetate), and rubidium (acetate), whereas respiratory inhibited mitochondria are *impermeable* to potassium (acetate) and rubidium (acetate) and permeable only to sodium (acetate). The data of Fig. 1 show that the relative rates of increase of osmotically active volume under the latter conditions are consistent with this view. More importantly, a semi-quantitative estimate of the permeability properties of the mitochondrion can be made from these data. For example, if we assume that the osmotically active volume of our mitochondria prior (while still in 0·25 M sucrose) to introduction into the alkali metal acetate medium was of the order of 0·5 μl per mg protein,[6] then the half time for maximal swelling of respiratory inhibited mitochondria in sodium acetate media should be of the order of 2 to 3 minutes. This value is in good agreement with the value of approximately 3·0 minutes derived from optical measurements as shown in Fig. 2. However, the half time for the same volume excursion in potassium* or rubidium acetate media is on the order of 100 minutes as shown in Fig. 2. Assuming that permeability to salt is the rate limiting factor in these experiments, one would conclude that the inherent passive membrane permeability of mitochondria to sodium (acetate) ions must be some 40 times greater than that for potassium or rubidium (acetate) ions. One of the major purposes of this communication was to determine whether exchange diffusion studies would yield information in agreement with this postulated relationship.

Distribution of ions in mitochondria isolated from 0·15 M sodium or rubidium acetate

In the previous experiments it was assumed on the basis of water movements that alkali metal salt had penetrated the mitochondria during swelling. The data of Table I provide direct evidence in support of this assumption in relation to swelling in a medium

* Under conditions of the experiment described in Fig. 2, the mitochondrial response in 0·15 M potassium acetate was indistinguishable from that of rubidium acetate.

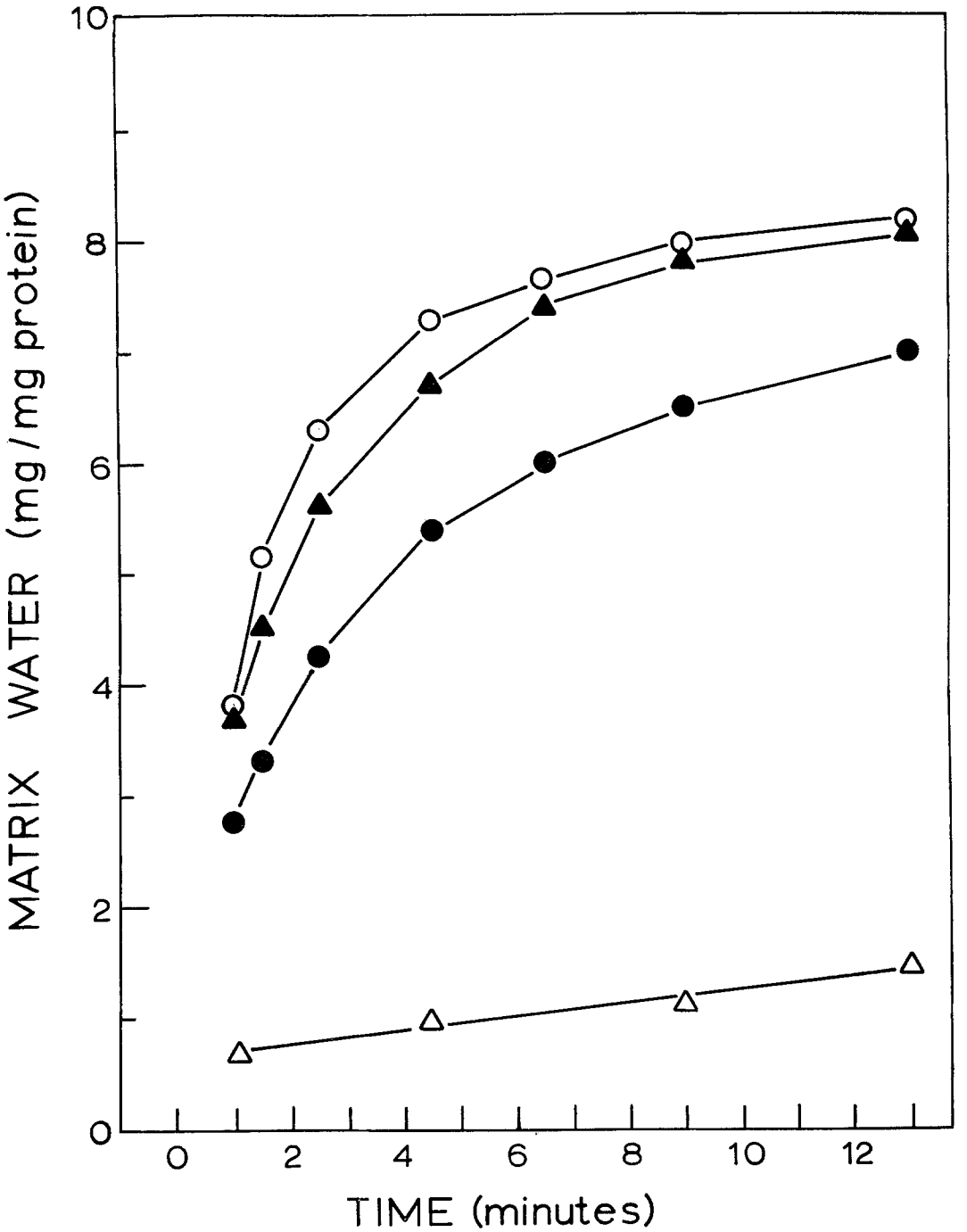

Figure 1. Time course for the increase in osmotically active volume of mitochondria incubated in a medium containing respectively sodium and rubidium acetate. Heavy beef heart mitochondria (0·5 ml of a suspension containing 20 mg of mitochondrial protein) was added to 9·5 ml of a medium containing sodium or rubidium acetate (pH 7·4) at a final concentration of 0·15 M. The incubation medium also contained ^{14}C-sucrose and where

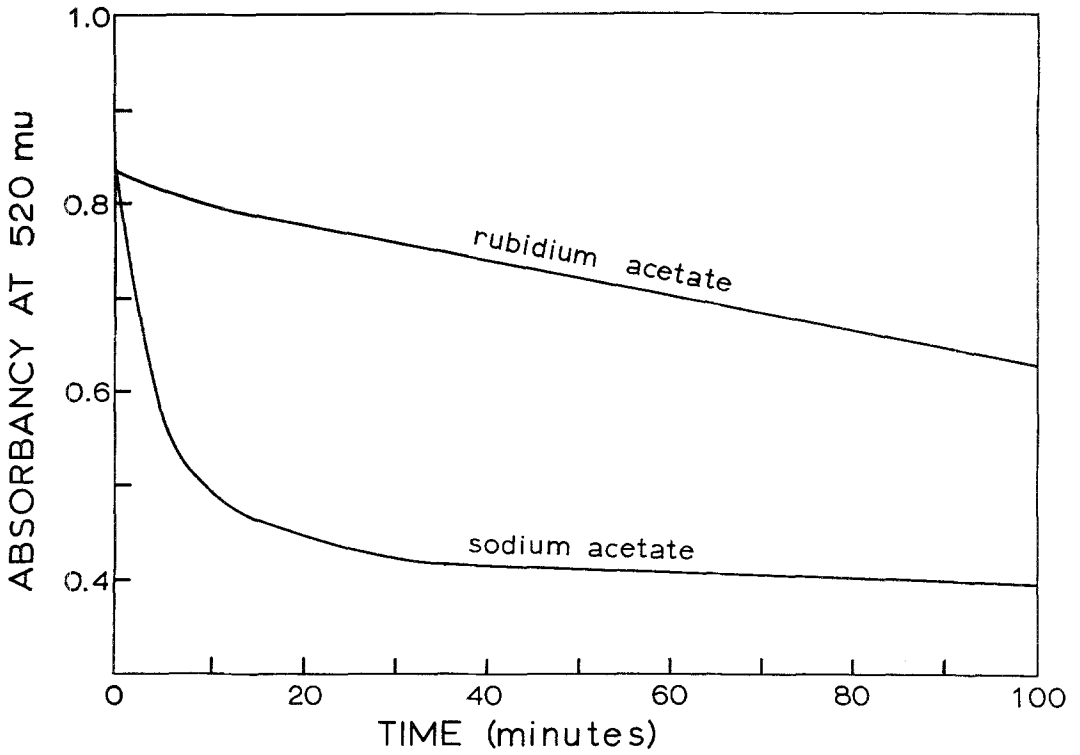

Figure 2. Time course for the decrease in absorbance at 520 mμ of mitochondria incubated in sodium and rubidium acetate. Heavy beef heart mitochondria (0·1 ml of a suspension containing 1·0 mg of mitochondrial protein) were added to 2·9 ml of a medium 0·15 M in sodium or rubidium acetate, pH 7·4, and containing antimycin and rotenone, each at a concentration of 2·0 μg per mg protein. Absorbance was measured at 25° as described in the section on methods. Under identical conditions the response of mitochondria to 0·15 M potassium acetate was indistinguishable from that of rubidium acetate.

0·15 M in sodium acetate. Under all conditions studied the concentration of sodium ions per μl of mitochondrial volume was in excess of the concentration present in the external medium. By contrast, the concentration of acetate ions in the mitochondrion was found to be consistently less than the concentration in the external medium. This behavior is of the type expected in a Donnan system, where the mobile anion ratio should be the inverse of the mobile cation ratio, i.e., $Na_i^+/Na_o^+ = Ac_o^-/Ac_i^-$. In the experiments described in Table I, these ratios are equal to 1·07 and 1·06 respectively in the absence of additions, and to 1·06 and 1·05 respectively in the presence of antimycin and rotenone. Furthermore, it is interesting to note, in the present case, that the sodium ion concentrations in the pellet are of the same order of magnitude as those reported previously for pellets derived from mitochondria suspended and incubated in sodium bromide media.[6] Since it is generally agreed that the mitochondrial membrane is

indicated antimycin and rotenone each at a concentration of 2·0 μg per mg protein. Tris-glutamate and Tris-malate (each at a final concentration of 2 mM) were present in all incubations. After incubation at 25° for the times indicated, duplicate 3·0 ml aliquots were sedimented and the pellets analysed as described in the section on methods. The water values expressed refer to sucrose-impermeable (matrix) water. The legends are as follows: open triangles—potassium acetate plus antimycin and rotenone, open circles—potassium acetate, closed circles—sodium acetate plus antimycin and rotenone, and closed triangles—sodium acetate.

TABLE I

Sodium and acetate ion content of sedimented pellets of mitochondria incubated in 0·15 M sodium acetate

Tracer ions	Additions	Water content of pellet (mg per mg protein)	Pellet ion content		Extent of equilibration of labelled ion between the medium and the mitochondrion[a]	
			Total mμmoles	mμmoles per mg pellet water	mμmoles per mg pellet water	mμmoles per mg protein
^{22}Na	None	12·97	2075	160·0	+10·0	+130
Ac-2-^{14}C	None	12·67	1800	142·0	− 8·0	−101
^{22}Na	AR[b]	9·97	1590	159·5	+ 9·5	+ 95
Ac-2-^{14}C	AR	9·72	1390	143·0	− 7·0	− 68

Heavy beef heart mitochondria (0·5 ml of a suspension containing 12 mg of mitochondrial protein) were added to 11·5 ml of incubation medium containing sodium acetate (pH 7·4) at a final concentration of 0·15 M. The media also contained either ^{22}sodium or acetate-2-^{14}C. Incubations were carried out for 15 minutes at 25°; 5·0 aliquots were centrifuged and the pellets analysed as described in the section on methods.

[a]This designation refers to the difference between the total measured ion content of the pellet and the product of the total pellet water content and the external salt concentration. A (+) designation means that the ionic concentration of the pellet water is greater than that in the suspending medium.

[b]Antimycin and rotenone were present at a final concentration of 2·0 μg per mg protein.

permeable to sodium (acetate), this finding lends support to our stated view[6] that the mitochondrial membrane is also permeable to sodium (bromide).

The results of parallel studies on the volume distribution of rubidium acetate are shown in Table II. It had been previously reported[6] that rubidium ion yielded similar volume distribution characteristics as did sodium ion when both were present as the chlorides. This similarity in behavior does not, however, extend to the acetate salts. While both rubidium and acetate ions are distributed in a Donnan fashion in actively respiring mitochondria, a totally different picture emerges in the presence of respiratory

TABLE II

Rubidium and acetate ion content of sedimented pellets of mitochondria incubated in a medium 0·15 M in rubidium acetate

Tracer ion	Additions	Water content of pellet (mg per mg protein)	Pellet ion content		Extent of equilibration of labelled ion between the medium and the mitochondrion	
			Total mμmoles	mμmoles per mg pellet water	mμmoles per mg pellet water	mμmoles per mg protein
^{86}Rb	None	11·95	1912	160	+10	+119·5
Ac-2-^{14}C	None	11·63	1664	143	− 7	− 81·5
^{86}Rb	AR	4·38	601	137	−13	− 52·6
Ac-2-^{14}C	AR	4·32	492	114	−36	−155·0

Except for the substitution of rubidium acetate for sodium acetate, and ^{86}rubidium for ^{22}sodium, the experimental details are identical with those described in the legend for Table I.

inhibitors as shown in Table II. For the first time, the concentration of rubidium ions in the mitochondrial pellet was found to be less (137 mM) than that present in the supernatant (150 mM). In agreement with this finding, the concentration of acetate under identical conditions was also found to be considerably less (114 mM) than in the presence of sodium (142–143 mM). Results of the kind shown in Table II are consistent with the view that the resistance to swelling of mitochondria in potassium or rubidium acetate is related to permeability and not, as in the case of the chloride,[6] to a lack of driving force.

Exchange diffusion of sodium and rubidium acetate ions

In the previous sections we have established (a) that actively respiring mitochondria swell rapidly in sodium, potassium, and rubidium acetate whereas mitochondria which are not respiring because of inhibitors swell rapidly only in sodium acetate media; and (b) that terminal ion analysis of mitochondrial pellets points to complete equilibration of alkali metal ions [Na^+(acetate) and Rb^+(acetate)] in actively respiring mitochondria and to complete equilibration only of Na^+(acetate) in respiratory inhibited mitochondria. Therefore, both the swelling characteristics and the terminal ion analysis suggest that non-respiring mitochondria are relatively impermeable to K^+ and Rb^+ (acetate). The data of Tables III and IV are in agreement with this view. The exchange diffusion experiments shown in these tables are of the same type previously described[6] in the studies on the permeability of mitochondria to alkali metal bromides. The only difference is that in the present experiments, mitochondria were allowed to swell under actively

TABLE III

Exchange diffusion of ^{22}sodium and acetate-2-^{14}C after incubation of mitochondria for 20 minutes in a medium 0·15 M in sodium acetate

Time of exposure to isotope (minutes)	Exchangeable Isotope: ^{22}Sodium		Acetate-2-^{14}C	
	mg water per mg protein	mμmoles ^{22}Na per mg pellet water	mg water per mg protein	mμmoles Ac-2-^{14}C per mg water pellet water
0·5	12·16	101	11·80	135
1·0	12·38	121	11·50	142
1·5	12·50	133	11·50	134
2·0	12·22	138	11·70	138
2·5	12·68	145	11·80	137
3·0	13·16	148	11·82	141
4·0	12·40	152	11·94	146
5·0	12·52	157	11·92	144
15·0	12·43 ± 0·21[a]	160·5 ± 3·5	11·35 ± 0·05	143 ± 4
20·0	12·67 ± 0·03	162·5 ± 1·5	11·49 ± 0·19	144 ± 3·5

Heavy beef heart mitochondria (0·2 ml of a suspension containing 10 mg of mitochondrial protein) were added to 4·73 ml of a medium 0·15 M in sodium acetate (pH 7·4). In addition, 50 μl of a solution of ^{22}sodium or acetate-2-^{14}C, and 20 μl of an ethanolic solution of antimycin and rotenone each at a concentration of 1·0 mg per ml, were added to the above mixture after 15 minutes of incubation. Duplicate 3·0 ml aliquots were centrifuged after exposure from 0 time for 15 and 20 minutes of incubation and the resulting pellets were analysed as described in the section on methods. The experiments indicated by time of exposure from 0·5 to 5·0 minutes were all centrifuged after 20 minutes of incubation.

[a]Average error of duplicate experiments.

TABLE IV

Exchange diffusion of ^{86}rubidium and acetate-2-^{14}C after incubation of mitochondria for 20 minutes in a medium 0·15 M in rudibium acetate

Time of exposure to isotope (minutes)	Exchangeable Isotope: ^{86}Rubidium		Acetate-2-^{14}C	
	mg water per mg protein	mμmoles ^{86}Rb per mg pellet water	mg water per mg protein	mμmoles Ac-2-^{14}C per mg pellet water
0·5	9·50	79·8	9·22	142
1·0	9·52	82·6	9·10	132
1·5	9·68	78·5	9·28	139
2·0	9·76	84·3	9·03	142
2·5	9·62	91·7	9·20	139
3·0	9·78	87·6	9·42	144
4·0	9·72	94·0	9·46	143
5·0	9·60	92·0	9·20	139
15·0	9·77 ± 0·03	161 ± 2·0	9·48 ± 0·08	146 ± 5·5
20·0	9·74 ± 0·04	160·5 ± 0·5	9·34 ± 0·02	144 ± 2·0

Except for the substitution of rubidium acetate for sodium acetate, and ^{86}rubidium for ^{22}sodium, the experimental details are identical with those described in the legend for Table III.

respiring conditions for 15 minutes at which time both the tracer ion and respiratory inhibitors were added and exchange diffusion followed for 5 minutes. The following information is derivable from these tables: (a) under all conditions studied there was little or no change in water content during the period of exchange diffusion, i.e., 15 and 20 minutes; (b) in media containing either the Rb^+(acetate) or Na^+(acetate), the exchange diffusion of acetate ion was instantaneous within the time limits of experimental execution; (c) the exchange diffusion of Na^+(acetate) was considerably slower than that of Na^+(bromide),[6] yet almost complete equilibration was achieved in 5 minutes; and (d) the exchange diffusion of Rb^+(acetate) is considerably slower than that of Na^+(acetate) under identical conditions, equilibration being only 59% complete after 5 minutes. These data were analysed on a semi-logarithmic plot (Fig. 3), together with data from similar experiments with ^{42}potassium acetate. When the reciprocal of the per cent of complete isotopic equilibration was plotted against time, the following relationships were apparent: (a) the line depicted by B was found to fit data from the experiments for both potassium and rubidium acetate. When this line was extrapolated to a point on the abscissa representing 90% isotopic equilibration, the intercept was found to be equivalent to 90 minutes. The half time for 90% isotopic equilibration should therefore be of the order of 45 minutes. This half time value is considerably smaller than the observed half time value for maximum swelling of 100 minutes under such conditions. However, the scatter of the experimental points is considerable in the present case and an error of this magnitude is within the present experimental accuracy. More importantly, however, the exchange diffusion rate of ^{22}sodium (line A) approaches 90% equilibration in 7·5 minutes which would indicate a half time of the order of 3 minutes which is in good agreement with the value from swelling data of 2·5 to 3·0 minutes. Therefore, it is

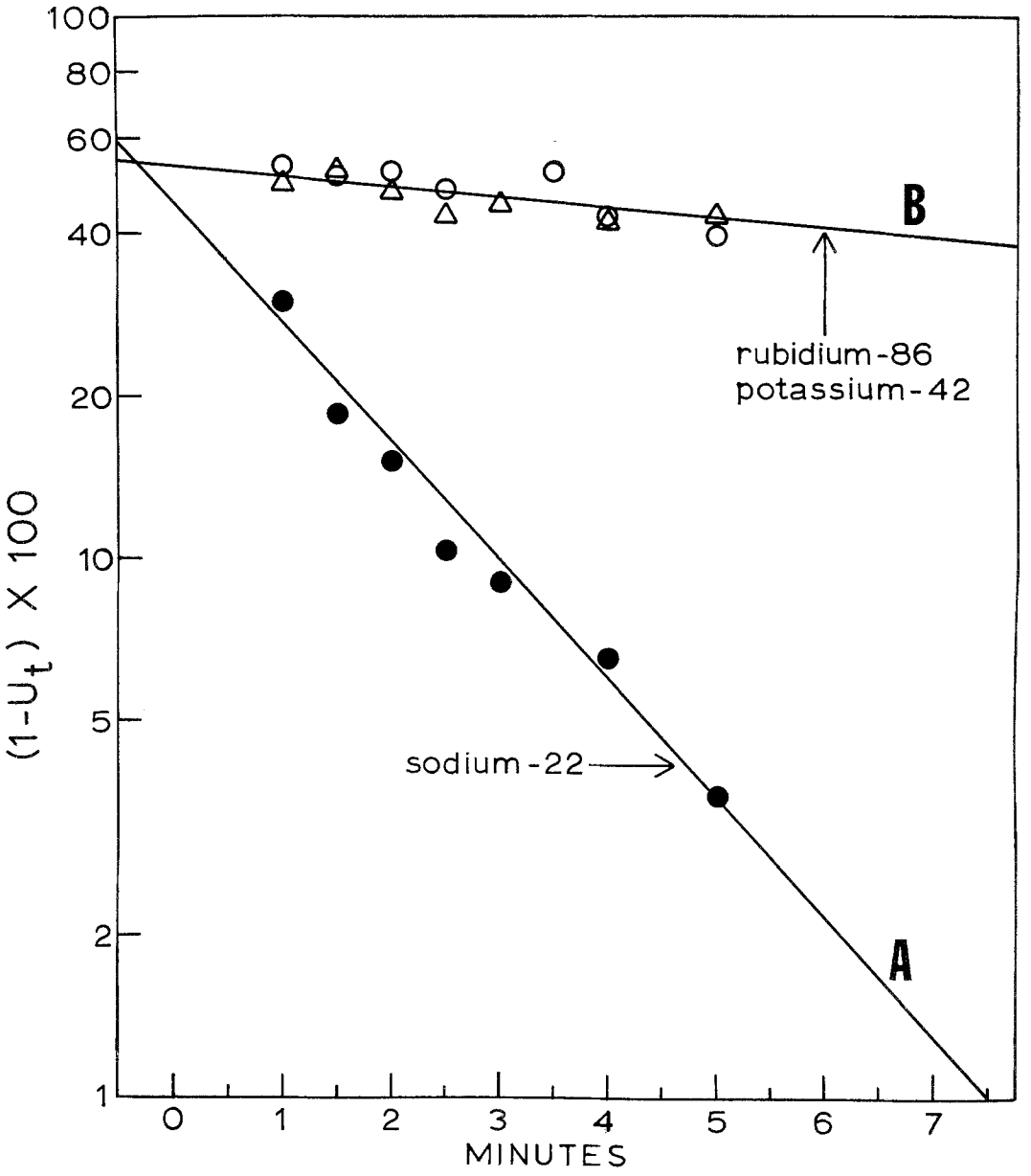

Figure 3. Kinetics of exchange diffusion of ^{22}sodium, ^{42}potassium, and ^{86}rubidium in a medium containing alkali metal acetate. Some of the data for this figure was obtained from Tables III and IV. In addition, an identical experiment was carried out with ^{42}potassium acetate. Line B represents data points for ^{86}rubidium (open circles) and ^{42}potassium (open triangles); line A represents the corresponding points for ^{22}sodium. (Ut) is the designation for the fractional attainment of isotopic equilibration.

apparent from these studies that the results of exchange diffusion are consistent with the swelling behavior of mitochondria in sodium, potassium, and rubidium acetate; and suggest that the rate of swelling of mitochondria in acetate media is limited by the rate of penetration of the cation.

Is the increased rate of energy-linked swelling in a medium containing K^+ or Rb^+(acetate) the result of a permeability change or of the energized translocation of rubidium or potassium ions?

Although non-respiring mitochondria appear to be relatively impermeable to rubidium and potassium acetate and therefore resist swelling under such conditions, actively respiring mitochondria swell extensively in rubidium and potassium acetate. Such a

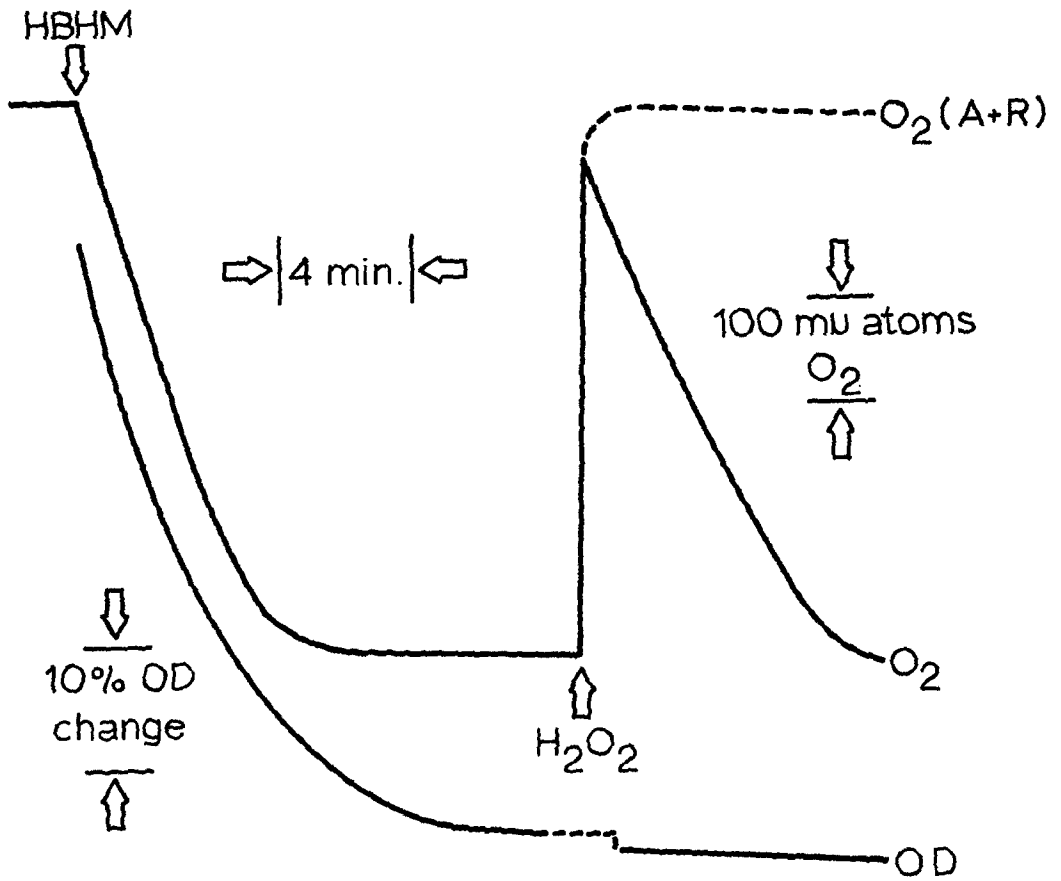

Figure 4. Oxygen consumption and absorbance changes during exchange diffusion studies of ^{86}rubidium in respiring and non-respiring mitochondria suspended in 0·15 M rubidium acetate. The experimental details are identical with those described in the legend for Table V. Absorbance measurements were made in a cuvette with a 0·1 mm path length (total volume 0·3 ml).

phenomenon can be rationalized in one of two ways. In the first place, it is possible that the energized mitochondrial membrane (i.e., energized by electron transfer or by hydrolysis of ATP) is permeable to rubidium or potassium acetate whereas the non-energized membrane (i.e., in the presence of respiratory inhibitors) is relatively impermeable to these salts. The second explanation would take the following form. The relative impermeability of the mitochondrial membrane to these salts as measured by unidirectional flux is unaffected by the energy state of the membrane, but rubidium or potassium acetate is actively translocated in the course of the energized swelling.

The experiments described in Fig. 4 and Table V represent an attempt to shed some light on this question. The design and rationale of the experiment may be described with the aid of Fig. 4. Mitochondria were introduced at 0 time into a medium 0·15 M in rubidium acetate, 3 mM each in Tris-glutamate and Tris-malate, and containing 300 μg catalase. The oxygen consumption and optical density (swelling) were simultaneously recorded for 15 minutes at which time the swelling was judged to be at least 90% complete. At time 15 minutes, 20 μl of a solution (0.27 M) of H_2O_2 containing ^{86}rubidium

TABLE V

Exchange diffusion of ^{86}rubidium in respiring and non-respiring mitochondria suspended in 0·15 M rubidium acetate

Time of exposure of isotope	Incubation time	Water content mg per mg protein	μmoles ^{86}Rb per mg protein	mμmoles ^{86}Rb per μl pellet water	%Isotopic equilibration
		Respiring			
0	15	12·05	0·915	76·0	47·0
3	18	12·87	1·23	95·5	59·0
6	21	13·58	1·53	112·5	69·5
15	15	12·73	2·06	161·5	100·0
18	18	12·95	2·11	163·0	100·0
21	21	13·48	2·18	162·0	100·0
		Non-Respiring			
0	15	13·1	1·04	79·4	49·0
3	18	11·88	1·14	96·2	58·3
6	21	13·0	1·39	107·0	66·1
15	15	12·27	1·98	161·0	100·0
18	18	12·3	2·02	165·0	100·0
21	21	12·3	1·99	162·0	100·0

Heavy beef heart mitochondria (0·6 ml of a suspension containing 30 mg of mitochondrial protein) were added to 14·35 ml of a medium containing rubidium acetate (pH 7·4) at a final concentration of 0·15 M, Tris-glutamate and Tris-malate, each at a final concentration of 3 mM, and 300 μg of catalase. After 15 minutes of incubation at 25°, 20 μl of a solution (0·27 M) of H_2O_2 containing ^{86}rubidium was introduced, and 3·0 ml aliquots were withdrawn at the times indicated for sedimentation and analysis according to the procedures described in the section on methods. In the respiring system, 30μl of an ethanolic solution of antimycin and rotenone (each at a concentration of 1·0 mg per ml) are added. The zero time exposure to isotope means that sedimentation was carried out immediately after addition of ^{86}rubidium.

was introduced and both oxygen consumption and optical density were recorded for an additional 6 minutes. Samples were withdrawn and sedimented at 15 minutes (immediately after addition of H_2O_2 and ^{86}Rb), at 18 minutes, and at 21 minutes. These were analysed as in the earlier exchange diffusion experiments. The control experiment was identical except that antimycin and rotenone was added to suppress oxidation just prior to the addition of peroxide and ^{86}Rb. There was yet another control in which the ^{86}Rb was present from the beginning of the incubation.

The results of this experiment are described in Table V. The most significant finding was that the rate of isotopic equilibration was almost identical whether the mitochondrial

population was energized or not. The very slightly faster rate under respiring conditions could be explained on the basis of active uptake of ^{86}Rb under the latter conditions, i.e. the difference between the 15 and 21 minute control experiments in respiring mitochondria was 2·18–2·06 or 0·12 μmoles ^{86}rubidium per mg protein, whereas these values remained unchanged in the non-respiring system (1·98 vs. 1·99 μmoles ^{86}Rb per mg protein).

Discussion

Previous attempts[6] to demonstrate a correspondence between the rate of passive swelling and the exchange diffusion rates of Na^+(bromide) and Rb^+ or K^+(bromide) led to the conclusion that the permeability of the mitochondrion to these ions was not the rate limiting step in the swelling process. On the other hand, the present investigation suggests a very close relationship between the rate of passive swelling and the exchange diffusion rates of Na^+ and Rb^+ (acetate). It is suggested that the resistance of mitochondria to passive swelling in media containing K^+(acetate) or Rb^+(acetate) is largely the result of the relative impermeability of the mitochondrion to these cations when paired with acetate. However, because the half time for maximum swelling in such media is still approximately twice as long as the half time for isotopic equilibration, it is still conceivable that resistance to configurational changes[12] and the lack of sufficient driving force also contribute to some degree to the lack of swelling in such media.

The finding of this investigation together with those of a previous communication[6] have enabled us to view the entire problem of mitochondrial permeability to small ions in a new light. In summary, the mitochondrial membrane is (1) rapidly permeable to chloride, bromide and acetate anions; (2) rapidly permeable to sodium potassium and rubidium cations when these are paired with chloride or bromide anions; (3) relatively impermeable to potassium and rubidium when paired with acetate and (4) relatively permeable to sodium ions when paired with acetate. The permeability behavior of the mitochondrion to alkali metal chlorides and bromides is not surprising since most synthetic and biological membranes share this behavior.[7–10] However, the passive permeability of the mitochondrion to sodium ions, in the presence of acetate co-ions, is almost 15 times greater than that for potassium or rubidium ions under identical conditions. This finding suggests that there exist, in mitochondria, a specific mechanism for the passive transmembrane movement of sodium ions in the presence of acetate ions, and further, that the principle of transmembrane cation movements is different in a medium containing chloride or bromide as anion than in a medium containing acetate as anion.

A theory for passive ion movements in mitochondria could be formulated as follows:

(a) *Mechanism I.* Ammonium or alkali metal ions paired to small anions, such as bromide and chloride, are freely permeable to the mitochondrial membrane systems.[6] Whatever the mechanism of permeation, the available evidence suggests that little discrimination exists between ammonium and other alkali metal ion components of the salt. This view is consistent with the observations of Bangham *et al.*[9] and of Papahadjopoulos and Watkins[13] that artificial phospholipid containing membranes are relatively permeable to alkali metal chloride and bromide ions with no significant difference for the cation series Li^+, Na^+, K^+, Rb^+, and choline. However, the synthetic lipid membranes[9,13] and certain biological membranes [e.g., red blood cell membrane,[16] nerve

cell membranes,[7] and muscle cells[17]] are relatively impermeable to larger* anions such as phosphate, acetate, and sulfate, and consequently impermeable to salts of these anions. In summary, therefore, the principles of ion permeation by mechanism I are accountable in terms of (1) a complete lack of discrimination between ammonium, choline and alkali metal cations, and (2) an apparent critical anion size (approximately equal to twice the hydrated anion radii of chloride or bromide) above which permeability to the salt is interdicted.

(b) *Mechanism II.* In the case of the mitochondrial membrane it is apparent that the above principles are not generally adhered to since our own findings as well as those of others[1–3] show that phosphate and acetate ions are rapid penetrants. Furthermore, when alkali metal ions are paired with phosphate or acetate co-ions, a heretofore unrecognized cation specificity emerges by which sodium ions are considerable more permeable than potassium or rubidium ions. It is, therefore, clear from these observations that the mechanism of transmembrane movement of alkali metal acetate or phosphate ions is based on a different set of principles from that of alkali metal chloride or bromide permeation by mechanism I. The essential points relative to permeation by mechanism II are (1) a significant degree of cation specificity by which Na^+ ions are at least 15 times more permeable than K^+ or Rb^+ ions, (2) an apparent indifference to the size of the anion (e.g. acetate = phosphate = propionate = phenylacetate), and (3) an absolute requirement for weak acid anions (i.e., anions possessing dissociable hydrogen ions in the neutral pH range).

When the anion component of a given salt exceeds a critical radius dimension (probably in the range of 4–5 Å) permeation by mechanism I is therefore interdicted and mechanism II is brought into play. The significant degree of cationic specificity associated with mechanism II is consistent with a carrier system. Recent investigations into the feasibility of isolating from mitochondria endogenous mobile ion carriers analogous to the microbiologically derived ionophores has in fact revealed the existence of a carboxylic acid substituted ionophore possessing complete specificity† for sodium ions in the presence of acetate co-ions.[18] The presence of the latter ionophore in beef heart mitochondria is entirely consistent with the much greater passive permeability of sodium (acetate) as opposed to rubidium or potassium (acetate). It is not unlikely that the permeability of weak acid anions is also mediated by carrier systems. Such systems have been postulated by others for acetate[2] and phosphate.[19]

The finding reported here that mitochondria undergo rapid energy dependent swelling in 0·15 M rubidium acetate while at the same time the rubidium ion flux at the steady state is essentially identical and relatively slow in both the energized and non-energized state provides strong evidence against the view that energization of the mitochondria increases its permeability to rubidium (acetate) or potassium (acetate). The rapid energy dependent swelling of potassium or rubidium acetate must therefore be explained by a mechanism unrelated to alterations in permeability. A reasonable mechanism would take the following form. M. Lee[15] has recently described an energy

* The data of Boyle and Conway[20] derived from ion mobilities in a uniform electric field suggests that the ionic radii of acetate, phosphate and sulfate ions are in the range of 1·8 to 2·3 times those of bromide and chloride ions.

† An earlier report from this laboratory[14] described the isolation of a neutral ionophore from mitochondria which did not possess appreciable specificity for sodium or potassium ions. Unlike the sodium specific carboxylic acid substituted ionophore, the neutral ionophore induced an active translocation of both sodium and potassium ions. However, the yield, specificity and qualitative activity of the neutral ionophore suggest that it is not involved in passive ion movements.

dependent binding of anion to the mitochondrial membrane which markedly increases the net fixed negative charge in the interior of the mitochondrion. Such a potential gradient (negative inside) would in theory accelerate the rate of entry of Rb^+(acetate) without affecting the permeability coefficient at steady state.

To some extent the data of Table II are consistent with this view since the mobile cation radio ($Rb_i/Rb_o = 1{\cdot}067$) exceeds the mobile anion ratio ($Ac_o/Ac_i = 1{\cdot}049$) under conditions where energized swelling is being driven by endogenous substrate. Such a finding is equivalent to saying that the mitochondrial pellet has a higher concentration of anion relative to the medium than would be predicted from Donnan theory on the basis of the mobile cation (Rb^+) distribution. Such an observation is consistent with the binding of anion according to the following equation:

$$Ac_{\text{bound}} = \left[[Ac_i]_{\text{obs}} - \frac{[Ac_o]}{[Rb_i/Rb_o]}\right] \times \frac{H_2O}{\text{mg protein}} = 27{\cdot}9 \text{ m}\mu\text{moles per mg protein}$$

Where Ac_i and Rb_i are the internal concentrations of acetate and rubidium ions, respectively, and Ac_o and Rb_o the external concentrations of acetate and rubidium, respectively.

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