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# ION TRANSPORT BY HEART MITOCHONDRIA

## XV. MORPHOLOGICAL CHANGES ASSOCIATED WITH THE PENETRATION OF SOLUTES INTO ISOLATED HEART MITOCHONDRIA

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SUMMARY

1. The morphology of isolated beef heart mitochondria has been compared with the water content, cation content, and mannitol-permeable water volume. Mitochondria suspended in isotonic potassium acetate take up $K^+$ and acetate spontaneously by an energy-linked reaction. The ion uptake is accompanied by extensive swelling of the mannitol (or sucrose)-impermeable water volume and by a dilation of the matrix space in electron micrographs. A similar ion uptake, increase in mannitol-impermeable water, and dilation of the matrix is seen in isotonic potassium acetate in the absence of energy when the membrane is made permeable to $K^+$ by the addition of gramicidin.

2. Expansion of the matrix and increases in the mannitol-impermeable water volume similar to those noted above for osmotic swelling in potassium acetate also occur when mitochondria are suspended in hypotonic solutions of sucrose or KCl.

3. In isotonic KCl the impermeant $Cl^-$ prevents the accumulation of $K^+$ either by the energy-linked reaction or by the passive, gramicidin-induced reaction. The uptake of $K^+$, increases in mannitol-impermeable water, and dilation of the matrix do not occur in this medium in the presence of a source of energy or in the absence of energy when gramicidin is added. Dilation of the matrix can be seen in isotonic KCl only when the membrane is made permeable to $Cl^-$ by either the addition of *p*-hydroxymercuribenzoate or by the combination of gramicidin and metabolic energy.

4. It can be concluded that the matrix space in electron micrographs correlates well with the mannitol-impermeable water volume which is observed in isotope experiments. It can also be concluded that the morphological changes reported here are closely related to osmotic factors.

## INTRODUCTION

In the previous communication in this series[1] it was established that the mannitol-impermeable space of isolated heart mitochondria increased markedly as a

function of both hypotonic swelling and osmotic swelling due to either the active or passive uptake of salts. In contrast, the mannitol-permeable space varied somewhat in size from preparation to preparation but remained relatively constant when any given preparation was subjected to large variations in total volume by osmotic forces. The present communication presents a study of the morphological changes which accompany these fluctuations in volume. The results are in substantial agreement with the recent conclusions of PFAFF *et al.*[2] that the matrix space of the mitochondrion represents the morphological equivalent of the mannitol-impermeable space, whereas much of the solute-permeable space can be accounted for by the intermembrane space. The relation of these changes in morphology which depend on ion uptake to those reported by HACKENBROOK[3,4] and by GREEN and co-workers[5–7] will be discussed. A preliminary report of a portion of this work has been presented[8].

## METHODS

The dual-label procedure for the evaluation of total water and mannitol-permeable water has been described in detail in the previous communication[1]. Swelling changes were monitored by changes in absorbance at 546 mμ using an Eppendorf photometer and a closed rectangular plexiglass cuvette with a magnetic stirrer. The cuvette contained a Clark oxygen electrode, combination pH electrode, Beckman cation sensitive electrode, and sampling port in an arrangement similar to that described by PRESSMAN[9]. The total volume of the cell was 8.5 ml. The mitochondria used were prepared from beef hearts by Nagarse digestion in the presence of ethyleneglycol-bis (β-aminoethylether)-*N*,*N*′-tetraacetic acid[10]. The cation content of isolated mitochondria was determined by atomic absorption spectrometry of acid extracts of the centrifuged pellets[11].

For a valid comparison of morphology and solute content it is necessary to fix the mitochondria rapidly and in a condition which corresponds to that of the centrifuged pellets which are to be analyzed. One procedure which we have used which appears to satisfy this condition can be outlined as follows: parallel incubations containing the identical unlabeled solutes as tubes for the dual isotope experiments were centrifuged at the same time as the penetration experiment. The penetration experiment is effectively stopped at a point in time when the supernatant is removed from the pellet, since no further penetration or exchange can then occur. The incubations for electron microscopy were also separated from the supernatant at this time and immediately covered with an identical charge of the supernatant containing osmium tetroxide (1 %). The pellets were broken up gently with a stirring rod and the fixation process continued for 1 h at 0–4°. Alternatively the pellets were immediately exposed to osmium vapors.

A number of experiments were also carried out using direct fixation of mitochondria in suspension by a procedure similar to that described by DEAMER *et al.*[12] employing in 1 % final concentration of neutralized glutaraldehyde.

In each case the fixed pellets were dehydrated in ethanol and embedded in epoxy resin[13]. Sections were cut using a Porter Blum microtome, picked up on 300-mesh uncoated grids and stained with uranyl acetate[14] (15 min) and lead citrate[15] (1 min) before examination in the microscope.

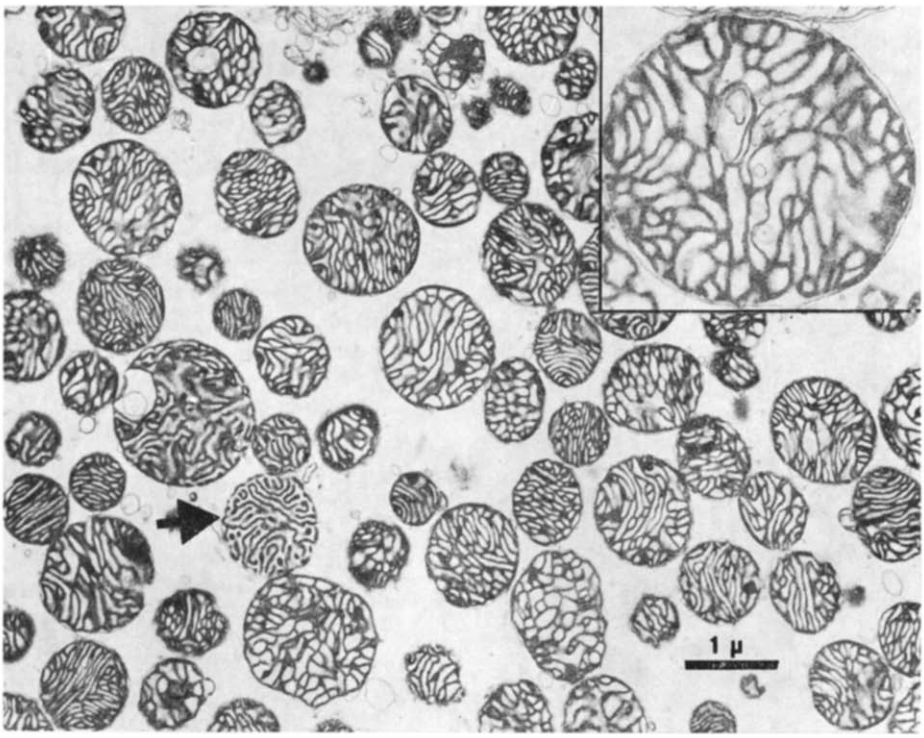


Fig. 1. Electron micrograph of beef heart mitochondria sedimented from cold 0.25 M sucrose and fixed with osmium tetroxide (× 12000). The mitochondrion indicated is in the 'energized twisted' conformation described by GREEN *et al.*[7]. The insert shows a higher magnification of a typical mitochondrion (× 30500) isolated and fixed under these conditions.

## RESULTS

### *Morphology of heart mitochondria in isotonic sucrose*

Fig. 1 shows a typical field of the preparation of heart mitochondria used in these studies. The preparation shown was fixed with osmium immediately after sedimentation from cold 0.25 M sucrose. Micrographs of preparations fixed with glutaraldehyde prior to osmium are almost indistinguishable from the field shown, as are fields from preparations which have been fixed in suspension by direct addition of glutaraldehyde prior to sedimentation. The matrix material is packed into a network consisting of sheets of darkly stained membranes which show numerous interconnections. All of the preparations used in this study were at least as homogeneous as shown in Fig. 1 before experimental manipulation. The cristae of these mitochondria in isotonic sucrose are clear and more or less vesicular in appearance. Most of the cristae do not show obvious connections to the intramembrane space which separates the inner membrane mass from the outer envelope. An occasional mitochondrion, such as the one indicated by the marker, is in the "energized twisted" configuration described by GREEN and co-workers[5–7], and in these particles the continuity of the intramembrane space and the cristae is quite apparent. These micrographs establish that the population of mitochondria examined in the following experiments is reasonably homo-

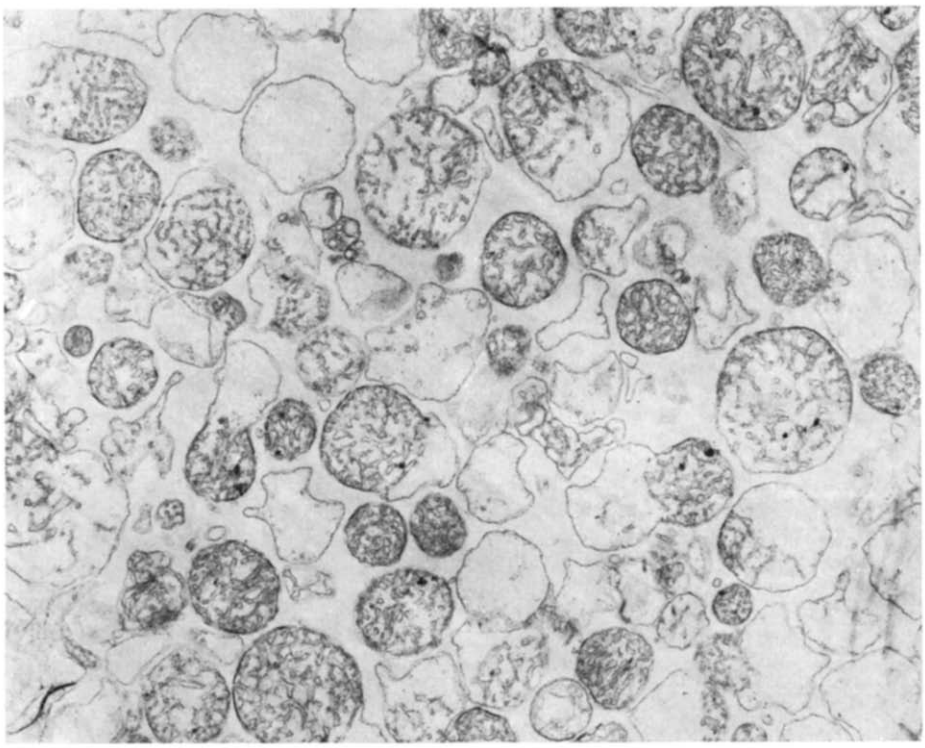

Fig. 2. Micrograph of beef heart mitochondria sedimented from 20 mM sucrose and fixed with osmium vapor (×6300).

geneous as isolated, and that the morphology closely resembles that of a number of preparations of beef heart mitochondria reported in the literature[7,16–20].

*Morphology of preparations of beef heart mitochondria suspended in hypotonic media*

Heart mitochondria swell when suspended in hypotonic solutions of sucrose, KCl, or potassium acetate in the absence of source of metabolic energy. The swelling can be followed by either the decrease in absorbance at 546 mμ or increased uptake of tritiated water[1]. The increase in water content is accounted for by an increase in the mannitol-impermeable space of the mitochondrion[1,2]. The most striking feature of electron micrographs of preparations suspended in hypotonic media is a marked increase in the size of the matrix network. The particles also appear to respond to quite different degrees to the stress of the hypotonic medium (Fig. 2) with the result that the fields are more heterogeneous. STONER AND SIRAK[20] have studied morphological changes of heart mitochondria as a function of decreasing osmolarity of the suspending medium and have shown that the matrix expands without an overall change in the volume of the mitochondrion during the early stages of this transition. As the osmolarity is decreased further, the expansion of the matrix causes extensive swelling of the particle with rupture of the outer membrane[20]. We have noted a similar transition in our own studies. The field presented in Fig. 3 shows the most typical forms seen in this transition. The form designated A is representative of the intermediate stage in which

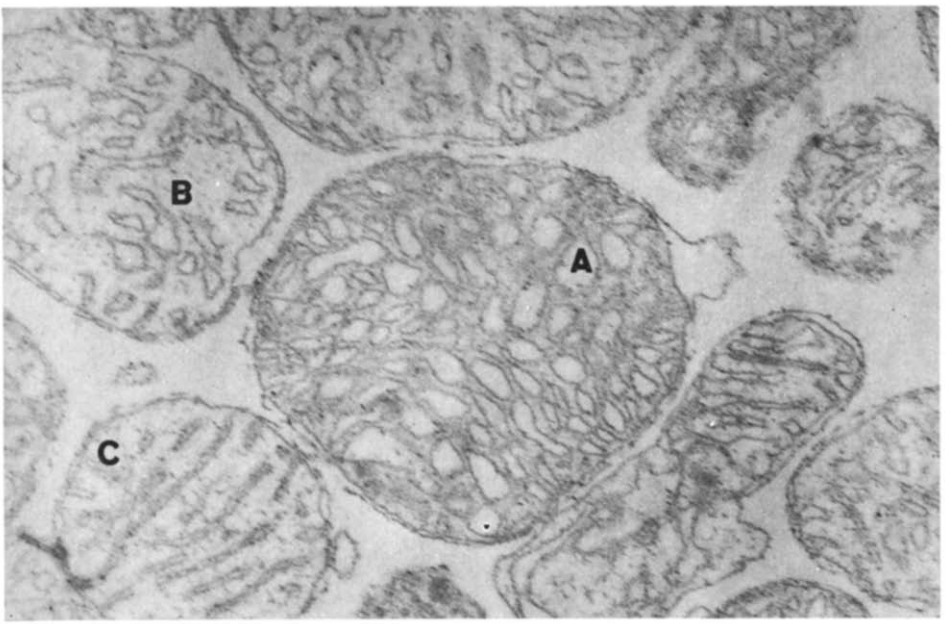


Fig. 3. Higher magnification (× 39900) of mitochondria isolated from hypotonic sucrose (50 mM) showing typical morphological forms. In form A the relation of the matrix network to that of mitochondria isolated from isotonic sucrose is clearly visible. Form B shows a massive increase in the matrix volume but retention of vesicular cristae. Form C shows a grossly swollen matrix but with parallel cristae.

the matrix network has started to dilate and become less dense. The relationship between the matrix and the cristae is clearly visible in such particles and the gross appearance of the inner membrane network has not been disturbed at this stage. However, as the matrix distends further and becomes even less dense, the forms designated B and C in Fig. 3 begin to predominate. The outer membrane of many of these particles is missing and two different alignments of the cristae can be distinguished. In the first of these the cristae are more or less vesicular (B), whereas in the second the cristae resemble two parallel membranes within the swollen matrix (C). Similar forms have also been reported recently in hypotonic suspensions of beef heart mitochondria by MUNN AND BLAIR[18] and by BLAIR AND TAN[19].

*Morphology of mitochondria suspended in isotonic potassium acetate*

Isolated heart mitochondria suspended in isotonic potassium acetate (0.12 M) in the absence of respiration contain about the same amount of total water and mannitol-impermeable water as corresponding suspensions in 0.25 M sucrose (*cf.* Table III of ref. 1). Fig. 4 shows a micrograph of a preparation sedimented under these conditions. The overall appearance of the field resembles that seen in 0.25 M sucrose except that the matrix network is aligned in much more parallel arrays and appears somewhat thicker than the corresponding membrane sheets in 0.25 M sucrose (*cf.* Fig. 1). As the incubation proceeds in the absence of energy there is little or no change in mitochondrial volume and no accumulation of $K^+$ (ref. 1). The morphology of the mitochondria also does not change during such an incubation.

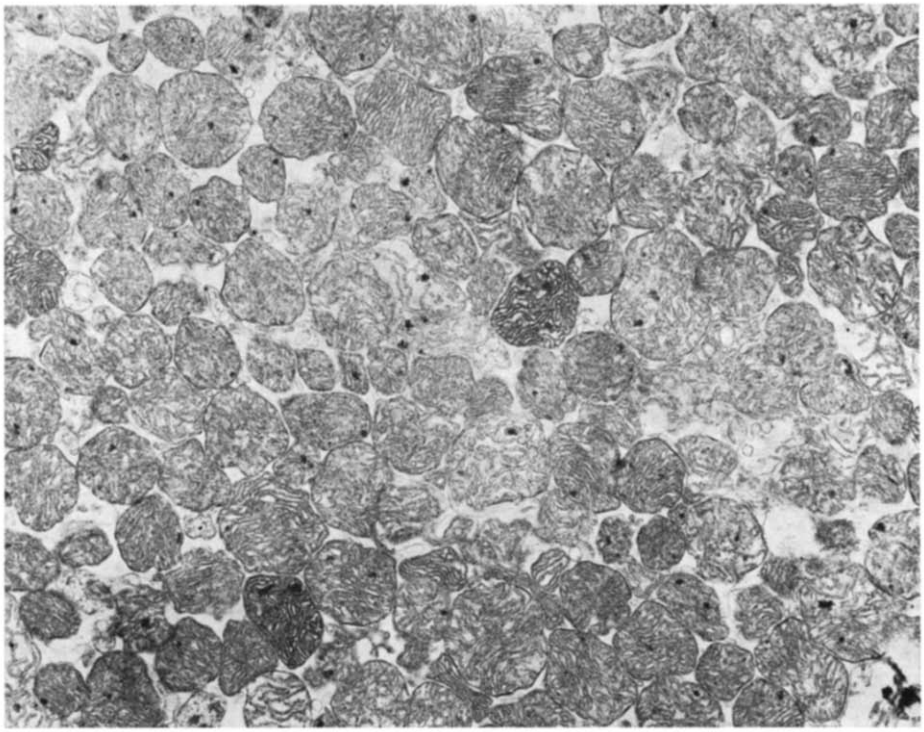

Fig. 4. Micrograph (×6300) of rotenone-treated mitochondria isolated from isotonic potassium acetate (0.12 M) and fixed with osmium tetroxide.

In the presence of a source of energy, such as endogenous respiration, the mitochondria swell by the criterion of decreased absorbance[10], increase in total water and in mannitol-impermeable water[1], and accumulate an amount of $K^+$ commensurate with an osmotic swelling process[1]. A typical experiment showing the changes in these parameters as a function of time is shown in Fig. 5. The appearance of a preparation sedimented after incubation for 2 min under these conditions is shown in Fig. 6. Many grossly swollen mitochondria are present and the bulk of the remainder show extensive dilation of the matrix. These particles often retain parallel cristae, however. Many mitochondria have the appearance of forms B and C in the field of hypotonically swollen particles (Fig. 3). Higher magnifications of such mitochondria (Fig. 7) permit the tracing of the inner membrane around the matrix and confirm that it is indeed the matrix which increases in volume and becomes less dense to produce the appearance typical of these osmotically swollen particles.

A similar swelling of the particles has been demonstrated to occur by light scattering criteria in the absence of energy in isotonic $K^+$ acetate when the mitochondria are treated with gramicidin to induce increased permeability to $K^+$ (ref. 10). The swelling can be accelerated by addition of an uncoupler of oxidative phosphorylation[10]. Under these conditions, as in the case of the energy-linked swelling just discussed, there is a marked increase in $K^+$ content, total water, and mannitol-impermeable water as a function of the time of incubation. Electron micrographs of mitochondria swollen

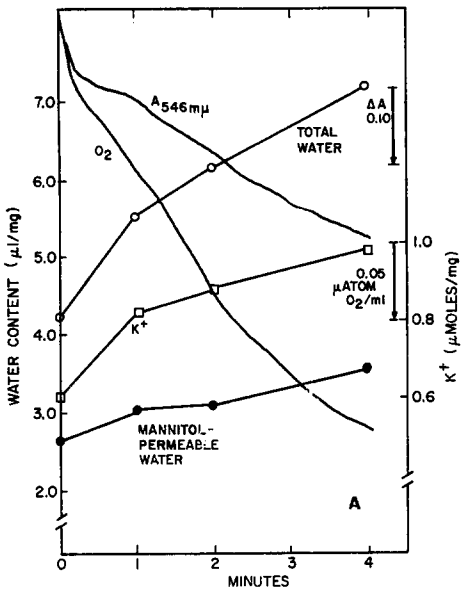


Fig. 5. Energy-linked swelling and $K^+$ uptake by mitochondria suspended in isotonic potassium acetate. The mitochondria (10 mg of protein) respired with endogenous substrate in a medium of potassium acetate (0.12 M), Tris-acetate (5 mM, pH 7.0), $^{14}C$-labeled mannitol (8 mM), tritiated water, and sucrose (15 mM added with the mitochondria). The reaction was monitored for $O_2$ uptake and change in absorbance at 546 m$\mu$ in the apparatus described in the text. At the indicated points parallel incubations were centrifuged rapidly in the Sorvall SE-12 rotor and analyzed for $K^+$, mannitol-permeable water volume, and total water as previously described[1]. Parallel samples were also fixed for electron microscopy as described in the text.

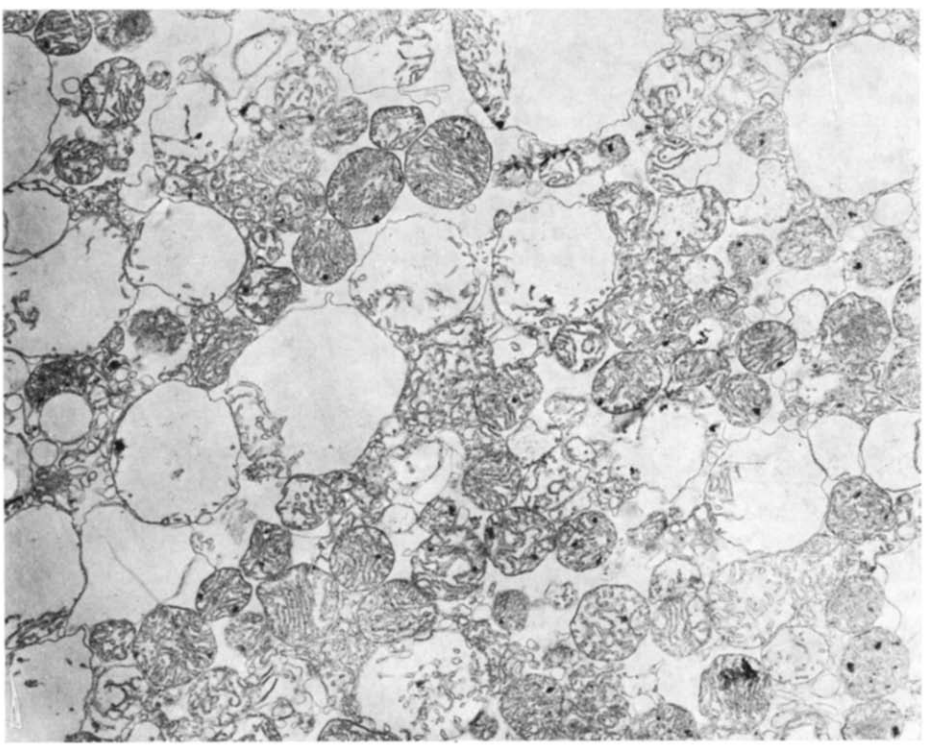

Fig. 6. Micrograph (×6300) of mitochondria after 2 min of incubation in isotonic potassium acetate under the conditions described in the legend for Fig. 5.

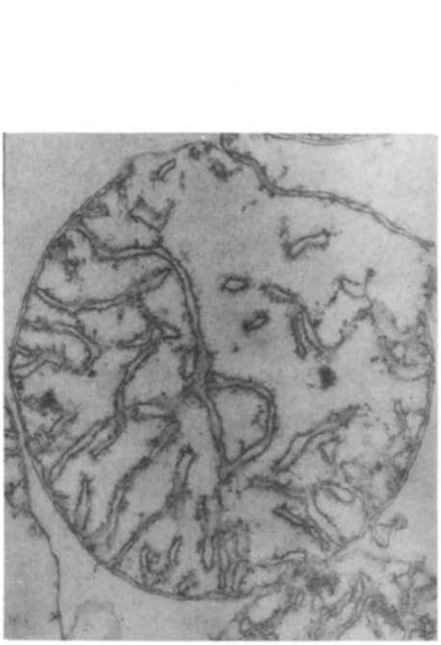

Fig. 7. Micrograph (× 28050) of a mitochondrion following energy-linked swelling and $K^+$ uptake from isotonic potassium acetate.

Fig. 8. Micrograph (× 18550) of heart mitochondria suspended in isotonic potassium acetate in the absence of a source of metabolic energy, but in the presence of gramicidin and carbonylcyanide *m*-chlorophenylhydrazone. The preparation was fixed by the addition of glutaraldehyde (1 % final) to the suspension after 5 min at 0°.

under these conditions (Fig. 8) show large numbers of grossly swollen particles and a number of intermediate forms. As in the case of energy-linked swelling, the matrix of the swollen particles is distended.

*Morphology in isotonic KCl*

Beef heart mitochondria suspended in isotonic KCl do not swell or accumulate $K^+$ in the presence of respiration[1,10], since ion uptake is prevented by the limited permeability of $Cl^-$ under these conditions[21,22]. The results of a typical incubation are presented in Fig. 9. The morphology of heart mitochondria suspended in this medium is similar to that seen in isotonic sucrose (Fig. 10). The degree of cross-linking of the matrix network is less in KCl than in sucrose. The cristae are larger and irregular in shape. This appearance is maintained throughout an incubation such as the one shown in Fig. 9. The field shown in Fig. 10 was obtained after 4 min of incubation in the presence of endogenous respiration, but fields which are indistinguishable from that of Fig. 10 are also found initially and at the intermediate points. Mitochondria incubated in isotonic KCl in the absence of energy contain the identical amount of $K^+$, total water, and mannitol-permeable water as preparations incubated with energy[1]. Fields from preparations incubated in the absence of energy also cannot be distinguished from Fig. 10. Mitochondria with identical morphology are also obtained when gramicidin and carbonylcyanide *m*-chlorophenylhydrazone are added in the

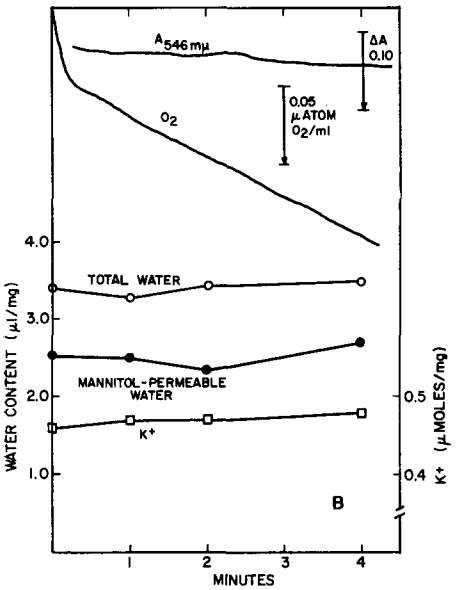


Fig. 9. Failure of respiring mitochondria suspended in KCl (0.12 M) to increase in volume or accumulate $K^+$. The experiment was carried out as described in the legend for Fig. 5 with the exception that chloride salts replaced the acetate.

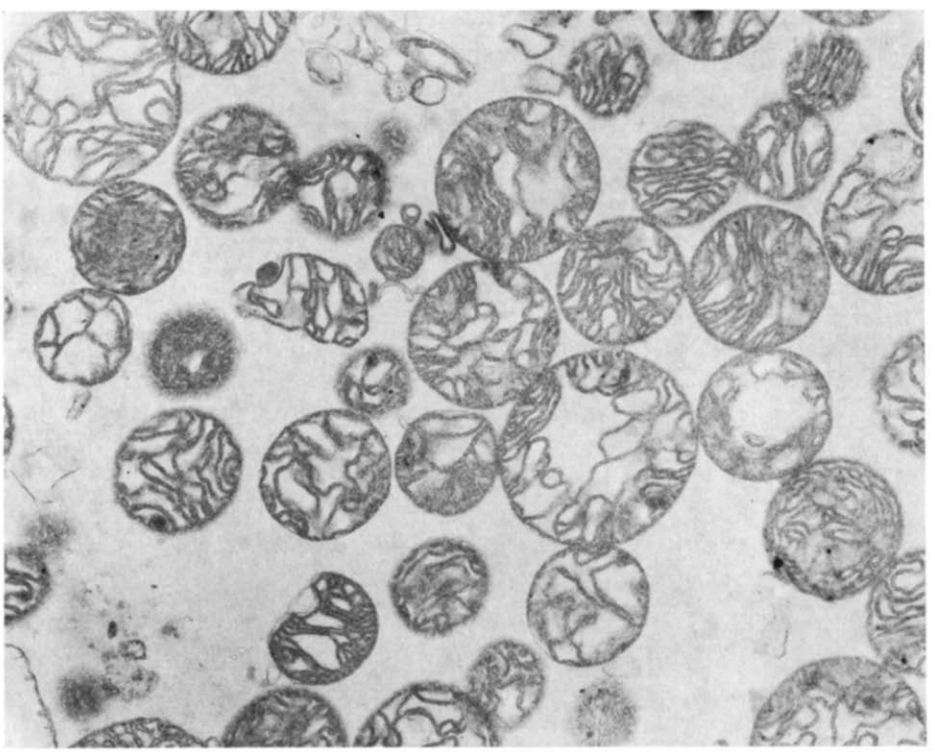

Fig. 10. Micrograph (× 17 100) of heart mitochondria fixed by direct addition of glutaraldehyde (1% final) to a suspension respiring with endogenous substrates in 0.12 M KCl.

absence of respiration to induce permeability to $K^+$. In this case also the limited permeability of the $Cl^-$ prevents passive osmotic swelling[1,10] and the matrix fails to swell, there is no $K^+$ uptake, and no increase in volume.

We have noted two conditions in which the accumulation of $Cl^-$ by heart mitochondria becomes appreciable, and in each of these cases which follow, accumulation of $K^+$, increased mannitol-impermeable water volume, and expansion of the matrix are readily demonstrated.

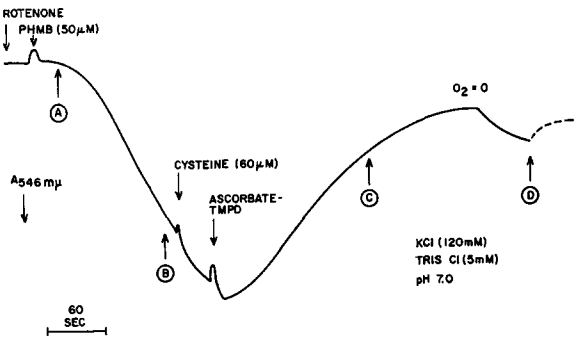


Fig. 11. Passive swelling and active contraction of heart mitochondria suspended in isotonic KCl and treated with *p*-hydroxymercuribenzoate (PHMB). Mitochondria (5 mg of protein) were added to 8 ml of a medium of KCl (0.12 M) and Tris–$Cl^-$ (5 mM, pH 7.0). At the indicated points rotenone (1 μg/mg), *p*-hydroxymercuribenzoate (50 μM), neutralized cysteine (60 μM), Tris–ascorbate (3 mM), and *N,N,N'N'*-tetramethylphenylenediamine (TMPD) (0.1 mM) were added to produce the changes in absorbance shown (*cf.* ref. 23). At the points indicated by the circled letters, neutralized glutaraldehyde was added directly to the cuvette to a final concentration of 1 %. The response to the addition of glutaraldehyde at point D is shown by the dashed trace. The morphological appearance at each of these points is shown in Fig. 12.

*Mercurial-induced swelling in isotonic KCl*

Passive swelling and accumulation of $K^+$ and $Cl^-$ can be induced in mitochondria suspended in isotonic KCl by the addition of nonpolar mercurial reagents, such as *p*-hydroxymercuribenzoate. A typical experiment is shown in Fig. 11 and a more complete discussion of the factors involved in this change in permeability has been presented elsewhere[23]. In the experiment shown glutaraldehyde was added at the indicated points to fix the mitochondria directly in suspension. Mitochondria fixed at point A (after addition of mercurial, but before swelling has commenced, Fig. 12A) show no change in morphology compared to untreated mitochondria (*cf.* Fig. 10). After the onset of swelling (point B of Fig. 11) the preparation shows extensive dilation of the matrix and deterioration of a portion of the mitochondria (Fig. 12B). This swelling can be reversed nearly quantitatively by the addition of cysteine and a respiratory substrate[23]. Samples examined by microscopy at a point close to the maximum observed contraction (Fig. 12C) show that large numbers of the mitochondria have returned to nearly the original appearance as the result of the energy-linked reversal of osmotic swelling. A few grossly swollen particles remain, and some debris from disrupted mitochondria is also visible. While some of the mitochondria in Fig. 12C show the densely packed matrix network typical of untreated preparations in KCl, the bulk of the preparation resembles the form designated 'A' in the micrograph of mitochondria swollen in hypotonic sucrose (Fig. 3). The matrix network is some-

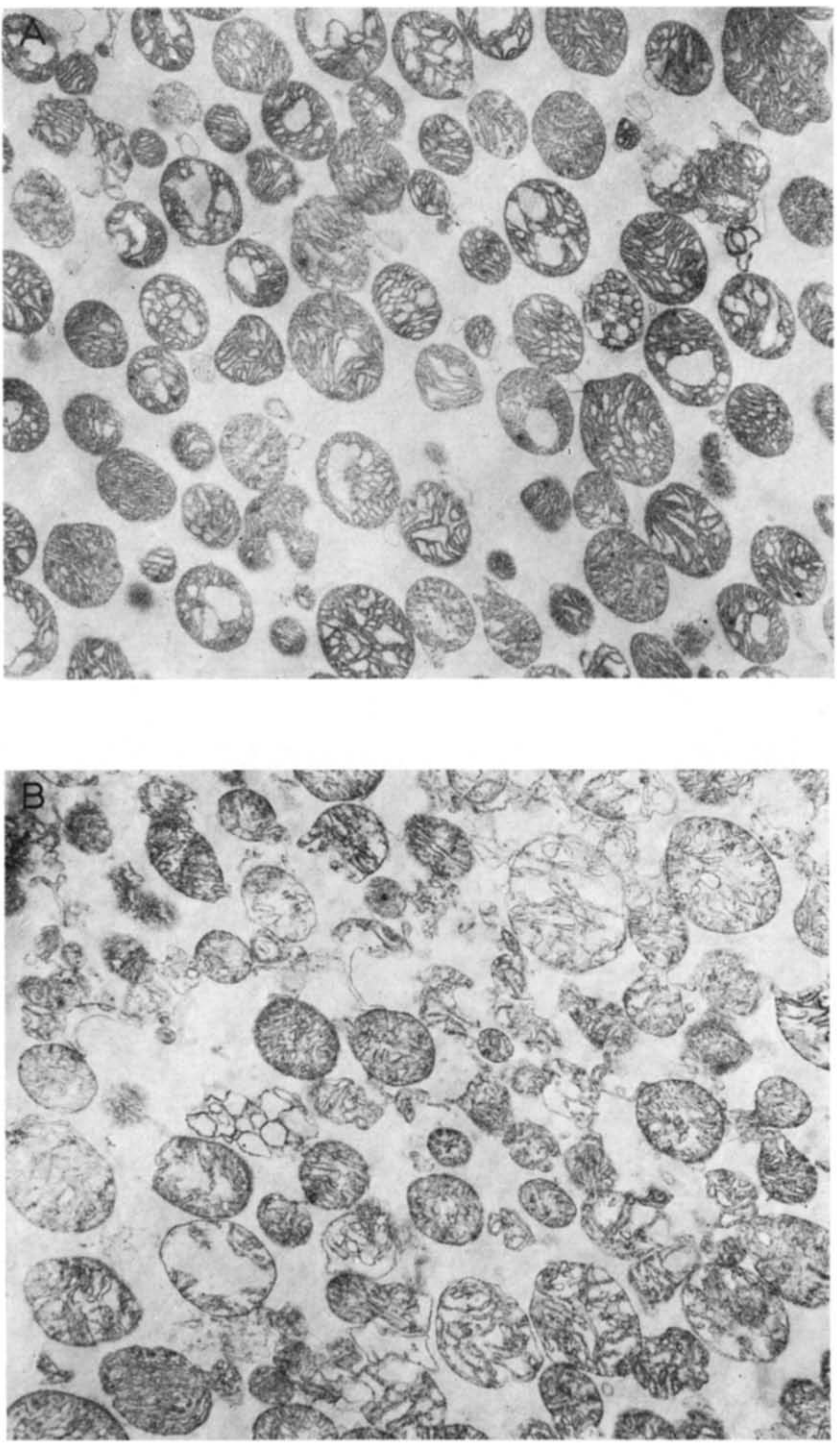


Fig. 12. For legend see next page.

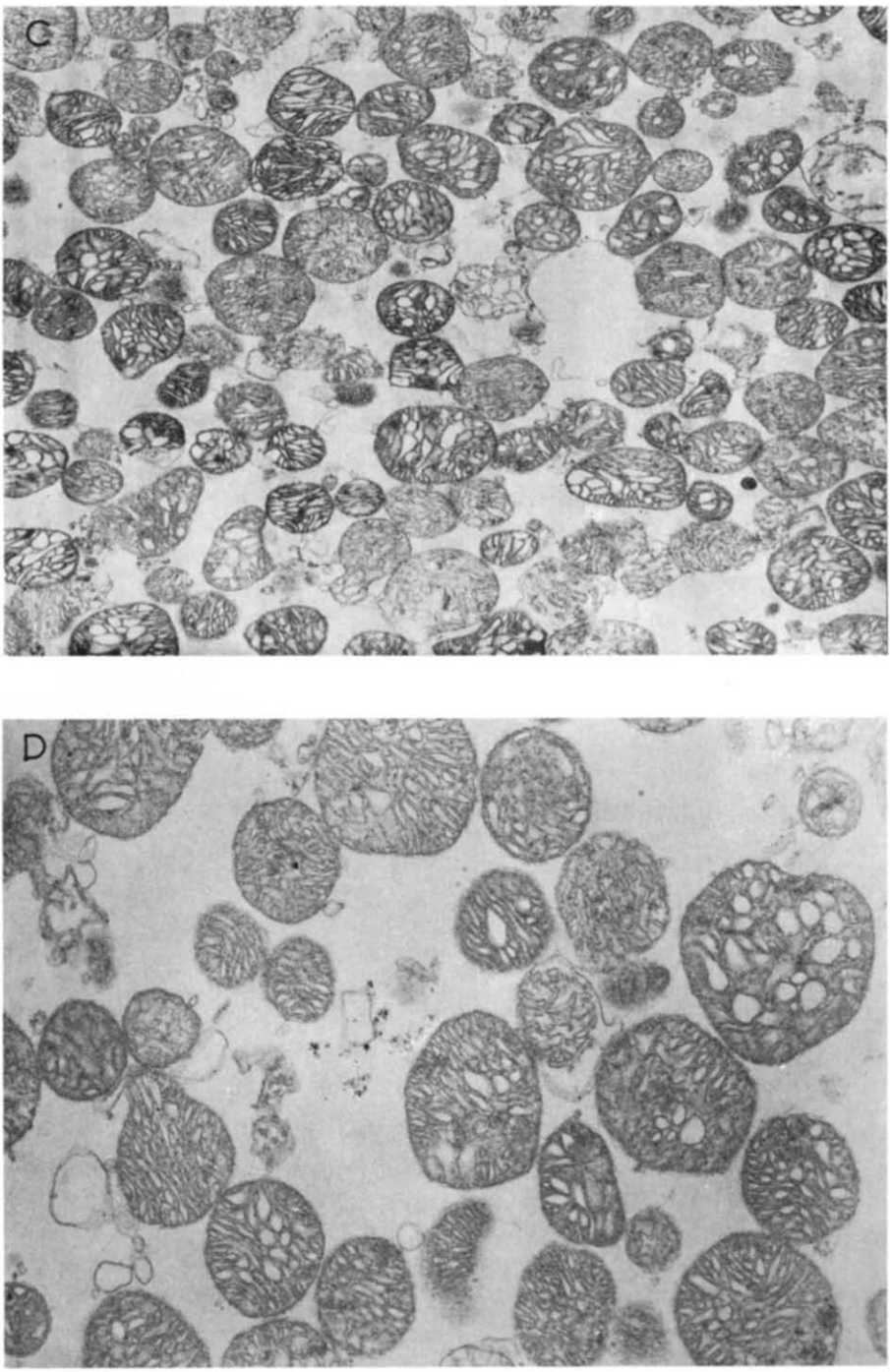


Fig. 12. Micrographs showing morphological changes which accompany the absorbance changes shown in Fig. 11. (A) ×10000 field from point A of Fig. 11. (B) ×10000 field from point B. (C) ×10000 field from point C. (D) ×20000 field from point D.

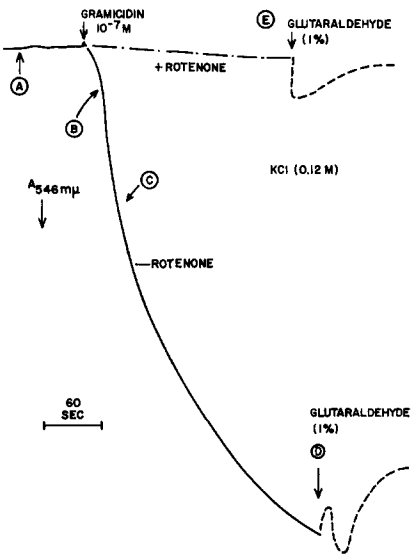


Fig. 13. Energy-linked swelling of heart mitochondria suspended in isotonic KCl and treated with gramicidin. The experiment was carried out as described in the legend for Fig. 11 with the addition of the indicated reagents. Glutaraldehyde was added to a final concentration of 1 % at the points designated by the circled letters. The response to glutaraldehyde addition at points D and E is shown by the dashed traces. The morphology at point C is shown in Fig. 14.

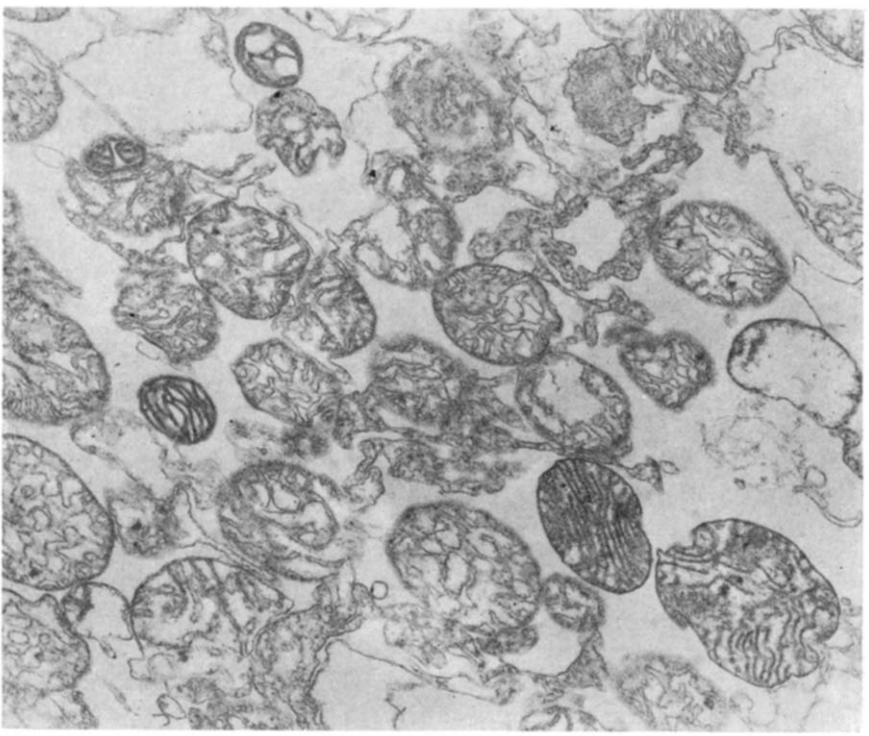

Fig. 14. Micrograph showing the morphology of the preparation at point C of Fig. 13 (× 17 100).

what expanded and diffuse in appearance. When the energy-linked contraction ceases due to anaerobiosis, a slow passive swelling commences[23]. Mitochondria examined at this stage of the reaction (Fig. 12D) show an even more extensive dilation of the matrix network which correlates with increased $K^+$ and $Cl^-$ content.

*Gramicidin-induced, energy-linked swelling in isotonic KCl*

Extensive accumulation of $Cl^-$ as well as $K^+$ has also been found to occur in mitochondria suspended in isotonic KCl when the combination of respiration and gramicidin is present (Fig. 13). As has been noted before, gramicidin in the absence of energy does not induce swelling in this medium, nor does respiration in the absence of gramicidin cause swelling (Fig. 9). An explanation for the extensive uptake of $K^+$ and $Cl^-$ which occurs under these conditions is still being sought. Regardless of the reason for the induced permeability to $Cl^-$ under these conditions, however, it is clear that both $K^+$ and $Cl^-$ accumulate, the volume increases, and as would be predicted from the results in acetate media, the matrix of the mitochondrion dilates (Fig. 14).

Other examples of osmotic swelling which result in a similar dilation of the matrix in electron micrographs include spontaneous passive swelling in isotonic ammonium acetate and in isotonic sodium acetate. The swelling in these cases resembles that reported here for potassium acetate and is therefore not presented. As expected, matrix expansion does not occur in the absence of energy in isotonic NaCl or $NH_4Cl$.

## DISCUSSION

The present studies confirm the conclusion of PFAFF *et al.*[2] and the brief report of BUTLER AND JUDAH[24] that the matrix of the mitochondrion is the morphological equivalent of the sucrose or mannitol-impermeable water volume of these organelles. Electron micrographs establish that the matrix expands and becomes less dense under conditions which result in an increased volume of the mannitol-impermeable compartment. These conditions include (a) hypotonic swelling in either sucrose, KCl, or potassium acetate media, (b) osmotic swelling due to the energy-linked accumulation of a cation and a permeant anion such as acetate, (c) passive osmotic swelling due to the induced accumulation of a cation such as $K^+$ in the direction of its concentration gradient when a permeant anion is present, and (d) passive osmotic swelling following the modification of the membrane by PHMB to induce permeability to both $K^+$ and $Cl^-$.

The close relationship between osmotic factors and the observed expansion of the matrix and increases in the size of the mannitol-impermeable compartment is further emphasized by the failure of either of these variables to change when the accumulation of ions is prevented by inability of either the anion or the cation to enter the osmotically responsive compartment. Swelling of the matrix and increases in the mannitol-impermeable compartment size do not occur when (a) a source of energy is available to support $K^+$ uptake, but only the nonpermeant $Cl^-$ is available as the counter ion, (b) the concentration gradient favors $K^+$ uptake, and the membrane is made permeable to $K^+$ by the addition of gramicidin, but no permeant anion is available to support swelling, and (c) the concentration gradient favors acetate accumulation, but no source of energy or ionophorous antibiotic is available to support the uptake of $K^+$.

In each of the above cases of swelling the process can be visualized as shown in the diagram (Fig. 15). In sucrose the densely stained matrix is interspersed with clear vesicular areas (cristae). In hypotonic media, water enters the matrix network causing it to expand, first to forms such as B and later to grossly swollen forms such as C. In the isotonic KCl and potassium acetate media the initial preparation shows a much

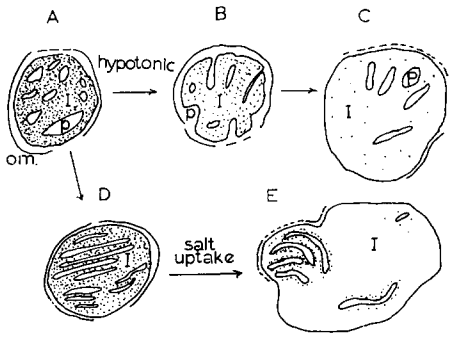


Fig. 15. Diagram of changes in morphology which result from hypotonic swelling and either energy-linked or passive osmotic swelling. The spaces labeled P indicate the mannitol-permeable space; those designated I, the mannitol-impermeable space. See the text for a more complete explanation.

higher proportion of parallel cristae as shown in D. Osmotic swelling, either active or passive, results in expansion of the matrix through intermediate stages to grossly swollen forms such as E in the diagram. In the diagram the matrix has been designated I, since this space correlates well with the mannitol-impermeable water volume. The intramembrane space has been labeled P in the diagram, since it appears to correspond to the mannitol-permeable space. The observation that large-amplitude swelling of mitochondria is the result of expansion and dilution of the matrix network agrees with the interpretation given in similar studies by a number of investigators[3,18,20,25–28].

We can also conclude from our studies that the bulk of the particles which swell osmotically are capable of reversing the swelling process. The swollen matrix network contracts to an appearance which closely resembles the configuration before the onset of the swelling cycle. The reversal shown in the mercurial experiments of the present study is energy-linked, but a careful study by STONER AND SIRAK[20] has also shown a similar contraction of heart mitochondria swollen in hypotonic sucrose or KCl and contracted osmotically.

Studies which attempt to relate the morphology of the mitochondrion to ion content, sucrose or mannitol permeability, and other parameters must take into account the possibility that the isolation of the mitochondria for analysis by centrifugation may cause changes in solute content and morphology. We have noted, however, that swelling in isotonic potassium acetate requires a continued supply of energy even though the movement of $K^+$ and acetate appears to be in the direction of the concentration gradient[10]. The packed pellets of mitochondria obtained in a rapid centrifugation should be nearly anaerobic and incapable of further swelling, if the indications obtained by absorbance studies follow under these conditions. In addition, since the concentration gradient is in the direction of ion uptake there should be little tendency for the mitochondria to lose ions to the medium and contract under these conditions.

Contraction at anaerobiosis or upon inhibition of respiration is not seen in absorbance experiments in this medium[10].

For a direct comparison of the analytical data obtained in these experiments with morphology it was felt that examination of preparations isolated in the same way and fixed as rapidly as possible after the isolation would provide the best data. A number of problems arise in this sort of protocol, including the presence of rather high concentrations of salt in the isolated pellets, the presence of large quantities of protein so that penetration of the osmium or glutaraldehyde may affect different portions of the pellet differently, the tendency of the centrifugation procedure to segregate lighter and heavier particles, and finally changes which result from the fixation process itself and from reagents such as buffers added with the fixative. The latter difficulty is of course inherent in all electron microscopy studies, but since each series of experiments reported was fixed under the same conditions, it is felt that the differences between the various treatments represent different states of mitochondrial morphology before fixation. In addition, direct osmium fixation, osmium vapor fixation, and glutaraldehyde fixation in solution give nearly the same picture of morphological alterations. Inhomogeneity within the pellet and in the reaction with the fixative cannot be eliminated, but can be minimized by sampling randomly and by examining a large number of fields. The conclusions and fields presented in this paper are the result of a large number of observations and are felt to be representative by such sampling criteria.

Studies which attempt to relate analytical data from centrifuged pellets to a continuously recorded parameter such as light scattering or oxygen uptake[2,3,7,12] are faced with the additional problem of possible changes which might result from the additional incubation time required for centrifugation and uncertainty regarding the metabolic condition of the particles during this operation. For this reason direct fixation in solution such as described by DEAMER *et al.*[12] is more useful in comparing morphology to the data of such traces. Fields obtained in this manner are somewhat more heterogeneous than the corresponding centrifuged pellets examined. Since the initial preparation examined in this way is homogeneous, this result indicates that a portion of the mitochondria are either predisposed to take up salt and swell in the saline media, or are predisposed to deteriorate in the presence of the fixative. It should be noted, however, that the bulk of the mitochondria in such preparation respond in qualitatively the identical way as has been noted for preparations fixed as pellets. Our conclusions therefore seem to be independent of these variables of fixation.

The close relationship between osmotic factors and the apparent size of the matrix network shown in the present study and that of DEAMER *et al.*[12] raises serious questions for investigators who consider changes in mitochondrial morphology to be the result of mechanochemical activity[2-7]. For example, GREEN and coworkers[5-7] have shown a thickening and rearrangement of the matrix network as a result of the addition of phosphate and a source of energy. These authors have suggested a conformational basis for energy conservation based on such observations. The present studies by no means disprove that energy conservation could be based on conformational changes as suggested, but the fact that permeant anions such as phosphate, substrates, and adenine nucleotides are used in the operations which bring about such morphological changes suggests to us that the major response seen in the electron microscope is an osmotic amplification of the molecular permeability of the membrane. It appears

possible that rather small changes in conformation at the level of the membrane lipid-protein array could bring about large changes in permeability to ions, which would in turn lead to changes in morphology as a result of osmotic swelling and shrinking.

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