ULTRASTRUCTURAL STUDIES OF BEEF HEART MITOCHONDRIA

III. The Inequality of Gross Morphological Change and Oxidative Phosphorylation

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ABSTRACT

The relationships between membranes and intramembrane compartments of isolated heart mitochondria are inadequately defined to express the induced morphological changes associated with the structural organization. The inner membrane and matrix are the major structural entities which undergo transformation upon alteration of metabolism or incubation conditions. To better express these morphological changes within a mitochondrion, two inner membranes *plus* enclosed matrix are defined as an inmerix (plural inmerices). Three general morphological forms of mitochondria can be distinguished by the size and shape of inmerices. These are distended, condensed, and coalesced inmerixal configurations. Hypotonic conditions and P_i in isotonic sucrose generate distended configurations. This P_i distention is apparently dependent on utilization of energy. It does not occur under anaerobic conditions. Oxidizable substrates generate condensed configurations. ADP and dADP generate coalesced configurations and stop formation of condensed and distended inmerixal configurations in the absence of inhibitors. ADP coalescence is apparently not dependent on an energy input. It occurs under aerobic and anaerobic conditions, and in isotonic and hypotonic media. Atractyloside completely inhibits the effects of ADP on inmerixal membranes whereas oligomycin does not. Distention by P_i is unaffected by the two inhibitors. Distended inmerices, without added P_i (12 mM and 62 mM sucrose), are coalesced by ADP. These studies indicate that coalescence of inmerixal membranes probably reflects the consequences of specific stoichiometric binding or translocation of adenine nucleotides.

INTRODUCTION

In 1964 Boyer (1) advanced a conformational change theory for the energy-conserving process of oxidative phosphorylation in mitochondria which may be derived from the "induced fit" model of Koshland (2, 3) in which protein conformation is changed by protein-substrate interaction.

The feasibility of a conformational change mechanism of oxidative phosphorylation has been investigated by Packer (4), Hackenbrock (5–7), and Green and coworkers (8–11). Electron microscopy was used by Hackenbrock as well as Green and associates to observe gross changes in morphology

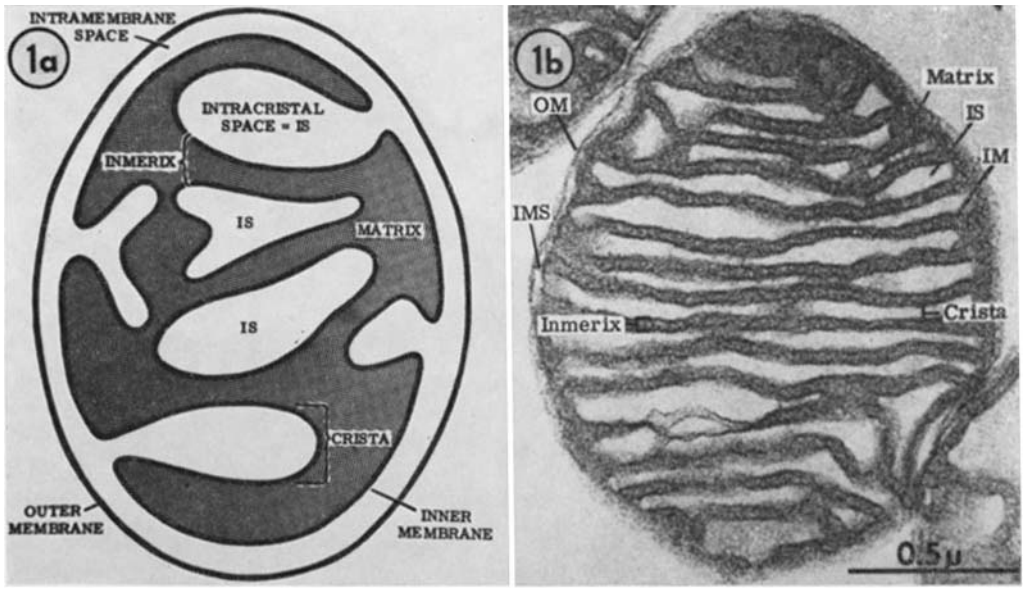

FIGURE 1 Morphological nomenclature of diagrammatic mitochondrion, (*a*), and sectioned, isolated beef heart mitochondrion, (*b*), showing classical relationships between membranes and intramembrane compartments. The new term inmerix is pictorially defined. × 45,000.

(configuration) upon addition of substrate, P_i, and ADP.[1] These configurational changes in membrane organization were assigned an energy-transducing role and were postulated to reflect the driving power of conformational changes at the molecular level. Additional evidence for the above mentioned interpretations came from observations on changes in low amplitude light scattering and optical density (12–16), which accompanied the "energy states" described by Chance and Williams (17). Reports (18–23) appeared previously which showed that energy transduction in the mitochondrion included not only oxidative phosphorylation and active transport but also mechanochemical processes. Other mechanochemical studies have shown that energy derived from ATP (24) or from electron transport (25, 26) can drive mitochondrial contraction.

Our initial experiments (27, 28) were designed to test the validity of the interpretation of the results obtained by Hackenbrock (5–7) and Green and associates (8–11). We were led to the conclusion that the ultrastructural changes observed by these authors reflected a binding of ADP to the inner mitochondrial membrane rather than an expression of the energy-transducing phenomenon as originally postulated. The present report further substantiates this argument. In addition, the importance of the inner mitochondrial membrane and the matrix it encloses was instrumental in naming this composite structure an inmerix; plural inmerices (29), (Fig. 1). The three basic configurations observed in sectioned specimens of isolated beef heart mitochondria were named the coalesced, condensed, and distended configurations in keeping with a morphological nomenclature (Fig. 2).

MATERIALS AND METHODS

Heavy beef heart mitochondria (HBHM) were isolated from the left ventricle of beef heart by a modification of the method employed by Crane et al. (30). Mitochondria were purified by utilizing the "light-heavy split" described by Hatefi and Lester (31). Mitochondrial protein was determined by the biuret method of Gornall et al. (32).

HBHM at 1 mg protein/ml in a 2 ml reaction

[1] *Abbreviations used in this paper:* ADP, adenosine diphosphate; dADP, 2′-deoxyadenosine-5′-diphosphate; ATP, adenosine triphosphate; dATP, 2′-deoxyadenosine-5′-triphosphate; P_i, inorganic phosphate; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; HBHM, heavy beef heart mitochondria.

mixture containing 0.25 M sucrose and 0.01 M Tris-HCl (pH 7.5) were incubated in a Dubnoff shaker for the electron microscopy experiments. Identical experiments were monitored with a Gilson Medical Electronics Inc. (Middleton, Wis.) Oxygraph, with the exception of anaerobic experiments. Stock solutions of pyruvate-malate and P_i were brought to pH 7.4 with KOH. The concentrations of these substances were pyruvate (15 mM), malate (1.5 mM), and P_i (15 mM) in all experiments, except where noted. Solutions of nucleoside mono-, di-, and triphosphates were neutralized to pH 7.0 with KOH and were added to make the reaction solution to 0.25 mM. Oligomycin in 95% ethanol when added was at a final concentration of 1 μg/ml. The atractyloside concentration (neutralized to pH 7.0 with NaOH) when employed was 25 μg/ml.

Anaerobic experiments were performed in a sealed Plexiglas glove box under a slight positive pressure of nitrogen. The sealed glove box was equipped with an oxygen electrode (Clark type) and a 10 ml jacketed reaction vessel with magnetic stirrer (the temperature was maintained at 30°C with an outside circulating water bath). Tygon tubing and tube connections were used for entry and exit of nitrogen, for circulating water, and for suction to rinse the reaction vessel. The glutaraldehyde-fixing solutions were bubbled with nitrogen for 3 min outside of the box and sealed. They were again gassed with nitrogen just before fixation of samples inside the sealed box. Reaction media containing all reagents except mitochondria and ADP were prepared on the bench, sealed, and placed inside the box. The reaction systems were bubbled with nitrogen 3–20 sec just before running an experiment. Only sufficient oxygen remained for the mitochondria to respire for 2–3 min before becoming anaerobic. Nitrogen was used to remove oxygen from the ADP solutions. Mitochondria were kept cold inside the box until used. Additions of mitochondria and ADP as well as removal of 1.0 ml samples were accomplished with Eppendorf pipettes (Brinkmann Instruments Inc., Westbury, N. Y.). The 1.0 ml reaction samples were injected into the anaerobic glutaraldehyde-fixing solutions.

Procedures for electron microscopy were essentially those of Green et al. (9) and D. W. Allmann (personal communication). The initial fixation employed 2% glutaraldehyde, 0.25 M sucrose, and 0.05 M sodium cacodylate, pH 7.5. This fixation was carried out on suspensions of mitochondria at the temperature of the experiment for 5–10 min. Then the suspensions were centrifuged (Spinco Model L, No. 40 rotor, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 20,000 rpm for 10 min at 4°C. Samples were washed at 0°C with 0.25 M sucrose *plus* 0.05 M sodium cacodylate, pH 7.5 and then fixed with 2% osmium tetroxide dissolved in 0.25 M sucrose plus 0.05 M sodium cacodylate, pH 7.5, for 1–2 hr at 0°C. The osmium-fixed fragments were placed in the wash solution for 15 min at 0°C. After removing the wash solution, the mitochondrial pellets were suspended in a solution which was 25% in ethyl alcohol and 1% in uranyl acetate for 15 min at 0°C. Pellets were then dehydrated with a series of graded alcohol solutions and propylene oxide and embedded in epoxy resin (Epon 812) prepared according to the procedure of Luft (33). Silver to silver gold sections were cut with a glass or diamond knife and placed on copper grids with a substrate film of collodion: prepolymerized methacrylate (F. Padgett, personal communication) and evaporated carbon. The specimens were stained with Reynolds' (34) lead citrate solutions and were examined using a modified RCA EMU-2B or EMU-3E electron microscope.

RESULTS

Morphological Nomenclature

Induced morphological changes associated with the structural organization of isolated heart mitochondria are inadequately defined and have not stressed the relationships between membranes and intramembrane compartments. Three basic morphological configurations shown in Fig. 2 were

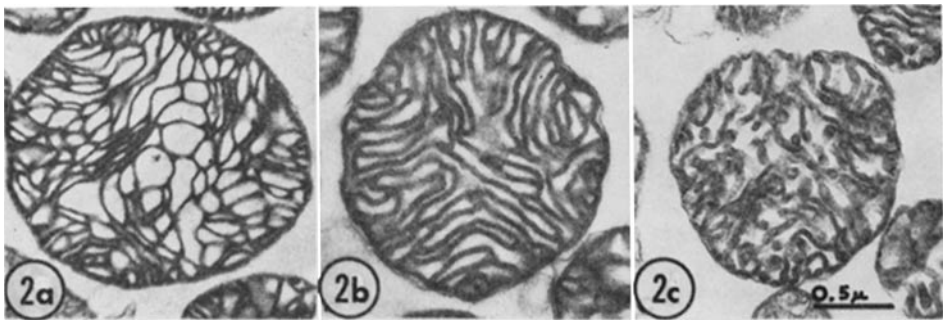

FIGURE 2 Three basic configurations of sectioned, isolated beef heart mitochondria. (*a*) coalesced and aggregated inmerices; (*b*) condensed inmerices; (*c*) distended inmerices. × 21,000.

originally named by Green and associates (8–11) as: (*a*) "nonenergized," (*b*) "energized," and (*c*) "energized-twisted." These terms became less meaningful during the course of our investigation. First, they are not morphological descriptions, they are hypothetical functional descriptions. Second, configurations assumed by mitochondria are not necessarily related to the energy state of the mitochondrion or even a particular steady metabolic state (27–29, 35, 36).

The major structural entity which undergoes morphological transformation upon alteration of metabolism or incubation conditions is the inner membrane matrix (29). The structure, in thin section, composed of two inner membranes plus enclosed matrix is defined as an inmerix. Fig. 1 shows the relationship of the inmerix as well as the classical terminology used in describing mitochondria. The three general morphological forms of mitochondria can be distinguished by the size and shape of inmerices and have been renamed the coalesced (aggregated form seen here), condensed, and distended configurations as seen in Figs. 2 *a*, *b*, and *c*, respectively.

The distended configuration (previously called the energized-twisted configuration) was the starting point for postulation of a new nomenclature. The inmerices of the mitochondrion in Fig. 2 *c* have a twisted tubular appearance at many points. Areas that appear swollen (distended) in many inmerices are almost circular in most cases, and many have inmerixal segments emanating from them which measure 300 A across. The disappearance or condensation of a majority of these distended areas led to the condensed terminology for the inmerices and therefore the configuration of the mitochondrion seen in Fig. 2 *b* (energized was the former term for this configuration). Segments of inmerices in both the distended and condensed configuration measure approximately 300 A across. Therefore the principal difference between these two morphological configurations is the disappearance of the twisted and distended inmerixal areas. The final morphology to be described is that of the coalesced configurations seen in Fig. 2 *a* (previously designated the nonenergized configuration). Again, the configuration is designated after assigning the term coalesced to the majority of the inmerices. This nomenclature was adopted because of the "coming together" of the inner membranes which is observed along many inmerixal segments. Note that this coalescence is in respect to inmerices (inner membrane plus enclosed matrix) and not cristae (inner membrane plus enclosed intracristal space). Furthermore, in Fig. 2 *a* another phenomenon is observable which in many cases accompanies the coalesced configuration. This is the phenomenon of aggregation in which the coalesced inmerices under go further ultrastructural rearrangement to give the observed honeycomb appearance. This has also been observed by other investigators (8–11). It must also be noted that aggregation is not limited to the coalesced configuration. Hunter and Brierley (37) have recently shown aggregation accompanying the condensed configuration. The expansion of inmerices in this case is apparently the result of ion accumulation.

P_i-Distended Inmerices

The level of P_i required to distend mitochondrial inmerices is shown in the electron micrographs in Fig. 3. It is apparent that the distended and twisted appearance of inmerices requires high levels of P_i ($\geq$ 5000 nmoles/mg, 5 mM). The effective level of adenine nucleotide (ADP or dADP) to produce an observable ultrastructural change was about 2 nmoles/mg protein (2 μM) or aproximately a 1000-fold lower concentration (27, 28). A gradual swelling of inmerices was observed as the phosphate concentration increased. It may also be noted that levels of P_i in the 100–500 μM range did not show significant numbers of distended mitochondrial profiles.

Adenine Nucleotides, P_i Distention, and Oligomycin or Atractyloside

Oligomycin and atractyloside are known inhibitors of oxidative phosphorylation, and their sites of action have been thoroughly investigated (38, 39). Fig. 4 compares the relative effectiveness of four adenine nucleotides in their ability to condense inmerices previously distended with P_i in the presence of either oligomycin or atractyloside. The first observation made was that neither oligomycin nor atractyloside prevented the P_i-induced distention (Fig. 4 *a*). Mitochondria in the presence of substrate, P_i, and atractyloside were indistinguishable from those in Fig. 4 *a*. The second observation is that only ADP and dADP produced an effect on the distended inmerices of mitochondria in the presence of P_i and oligomycin (Figs. 4 *b* and 3) whereas the triphosphates showed no observable changes as seen in Figs. 4 *c* and *d*. The change in

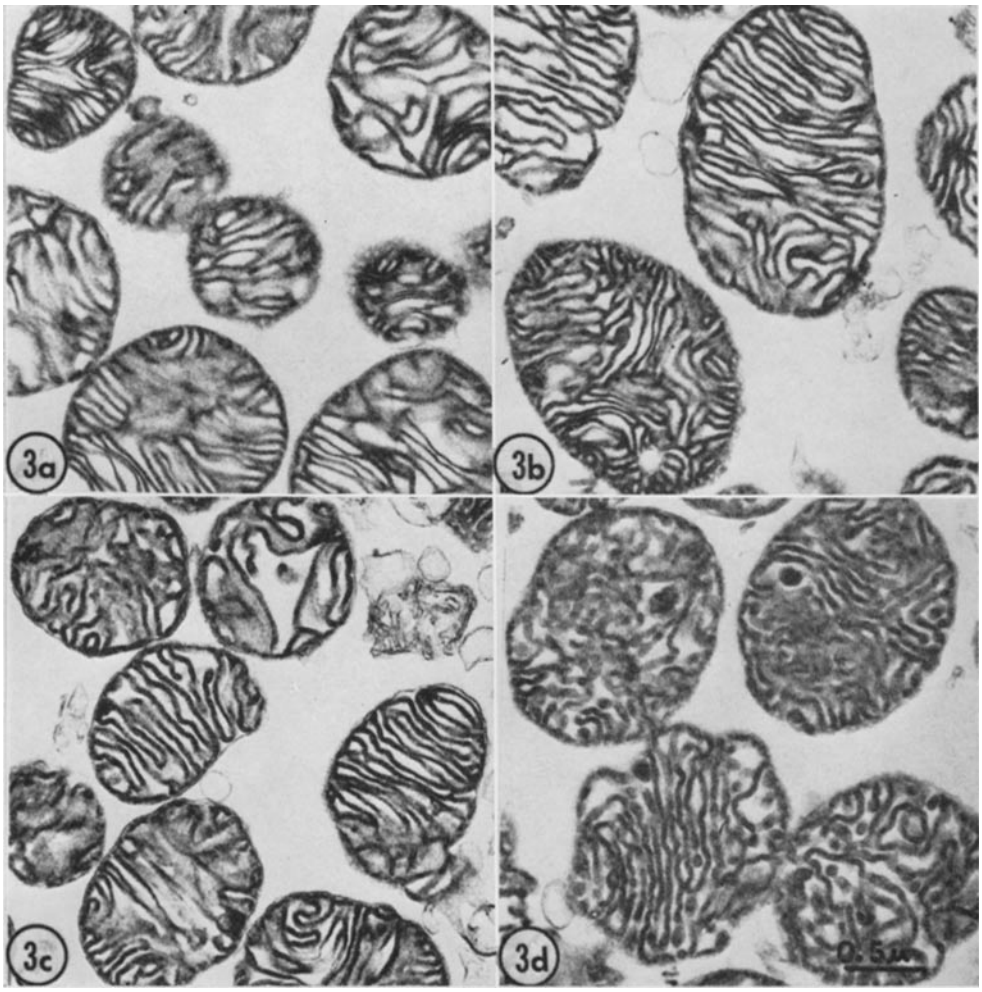

FIGURE 3 Induction of distended inmerices at 30°C. P_i concentrations are expressed in nmoles/mg protein. All reactions were run in the presence of substrate for 1 min, followed by the addition of P_i at the concentration given below for 1 min. (*a*) P_i (100 nmoles); (*b*) P_i (300 nmoles); (*c*) P_i (500 nmoles); (*d*) P_i (5000 nmoles). × 22,000.

configuration generated by ADP or dADP in the presence of P_i and oligomycin is to the condensed configuration. The final observation is that atractyloside inhibited the effects of ADP or dADP on distended inmerices (Fig. 4 *f*).

Anaerobiosis and Configuration

The electron micrographs in Figs. 5 and 6 show transformations in mitochondrial morphology in and oxygen-depleted environment. Depletion of small amounts of high energy compounds such as ATP may be rapid under anaerobic conditions. This suggestion has come from studies on rat liver mitochondria (40, 41). Other reactions which depend on the oxidized coenzymes nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN) are also inhibited because the ratio of reduced : oxidized coenzyme increases when an oxidizable substrate is present. The electron transfer chain is the primary source of reoxidation of the coenzymes and it can no longer accept reducing equivalents when the terminal electron acceptor, oxygen, is depleted. This environment should be ideal for testing ADP coalescence of inmerices be-

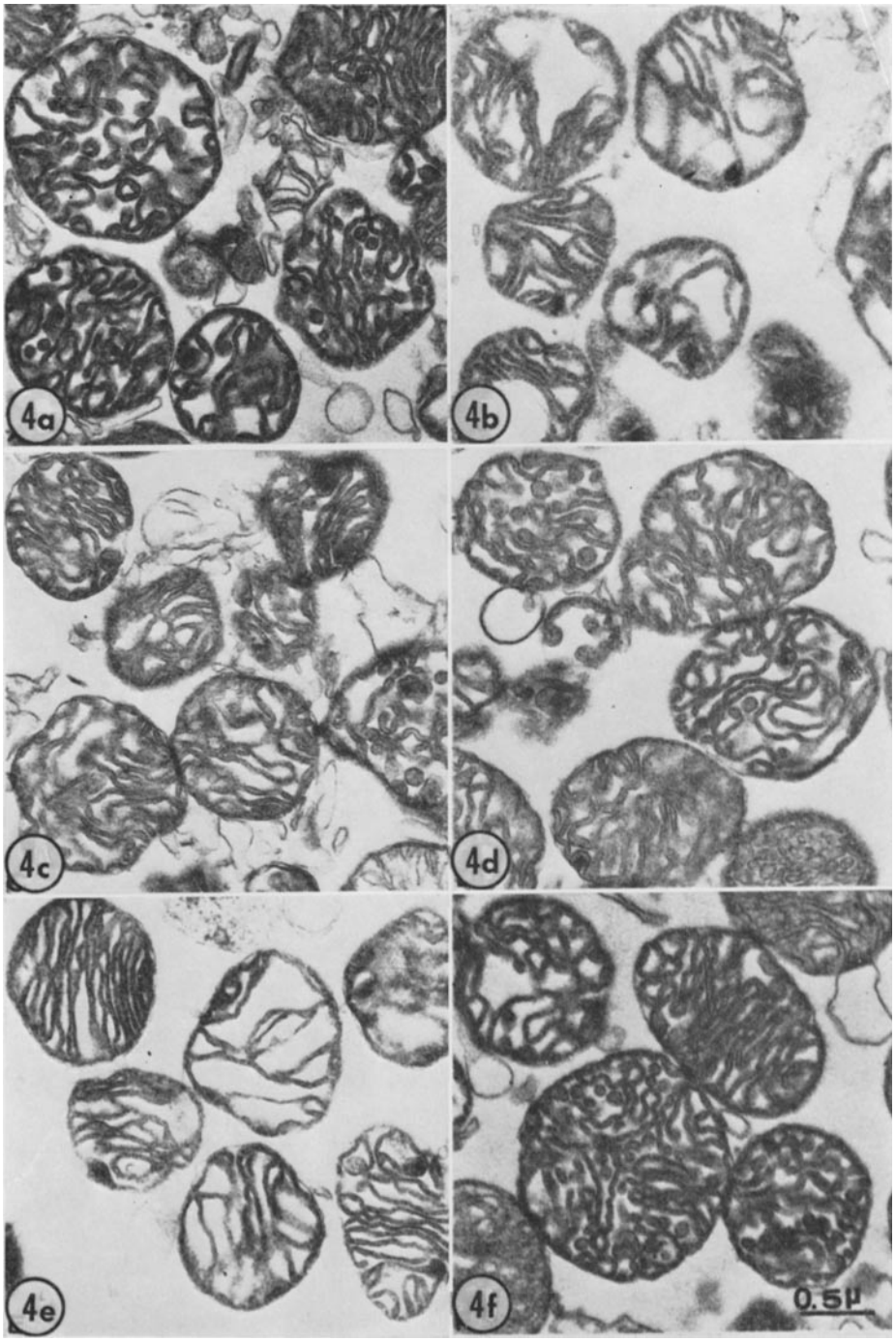

FIGURE 4 Effects of oligomycin and atractyloside on condensation of mitochondrial inmerices by adenosine di- and triphosphates in the presence of substrate and P_i. All experiments were run at 30°C with oligomycin or atractyloside for 1 min, followed by the addition of adenine nucleotide for 15 sec. (*a*) oligomycin; (*b*) oligomycin then dADP; (*c*) oligomycin then ATP; (*d*) oligomycin then dATP; (*e*) oligomycin then ADP; (*f*) atractyloside then ADP. × 20,000.

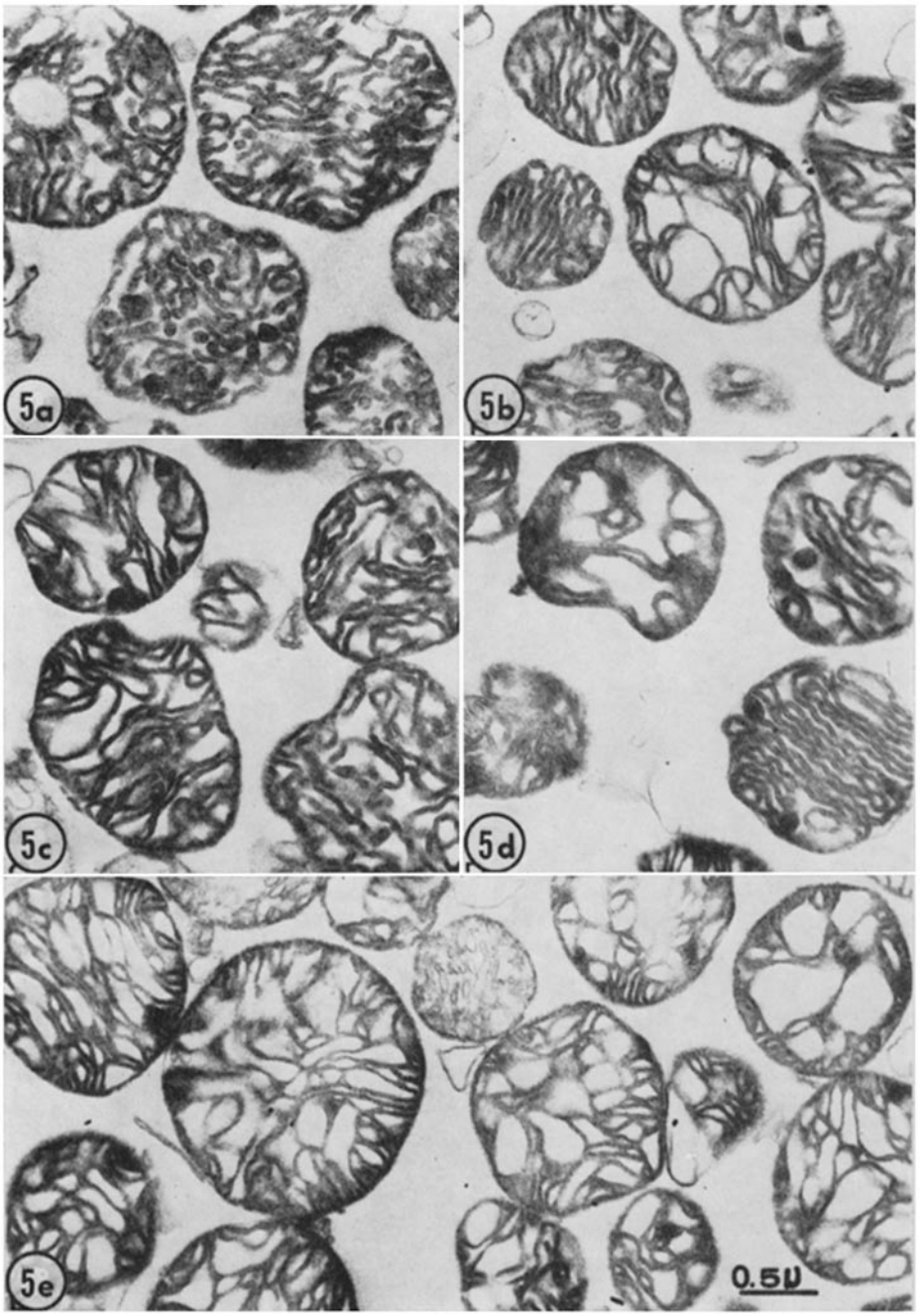

FIGURE 5 ADP-induced coalescence of mitochondrial inmerices under anaerobic conditions in the presence of substrate and P_i. Other conditions included 4 ml reaction medium with 1 mg protein/ml and ADP (500 nmoles/mg protein). (*a*) aerobic 1 min; (*b*) anaerobic 30 sec; (*c*) anaerobic 1 min; (*d*) anaerobic 2 min; (*e*) anaerobic 30 sec then ADP 30 sec. × 21,000.

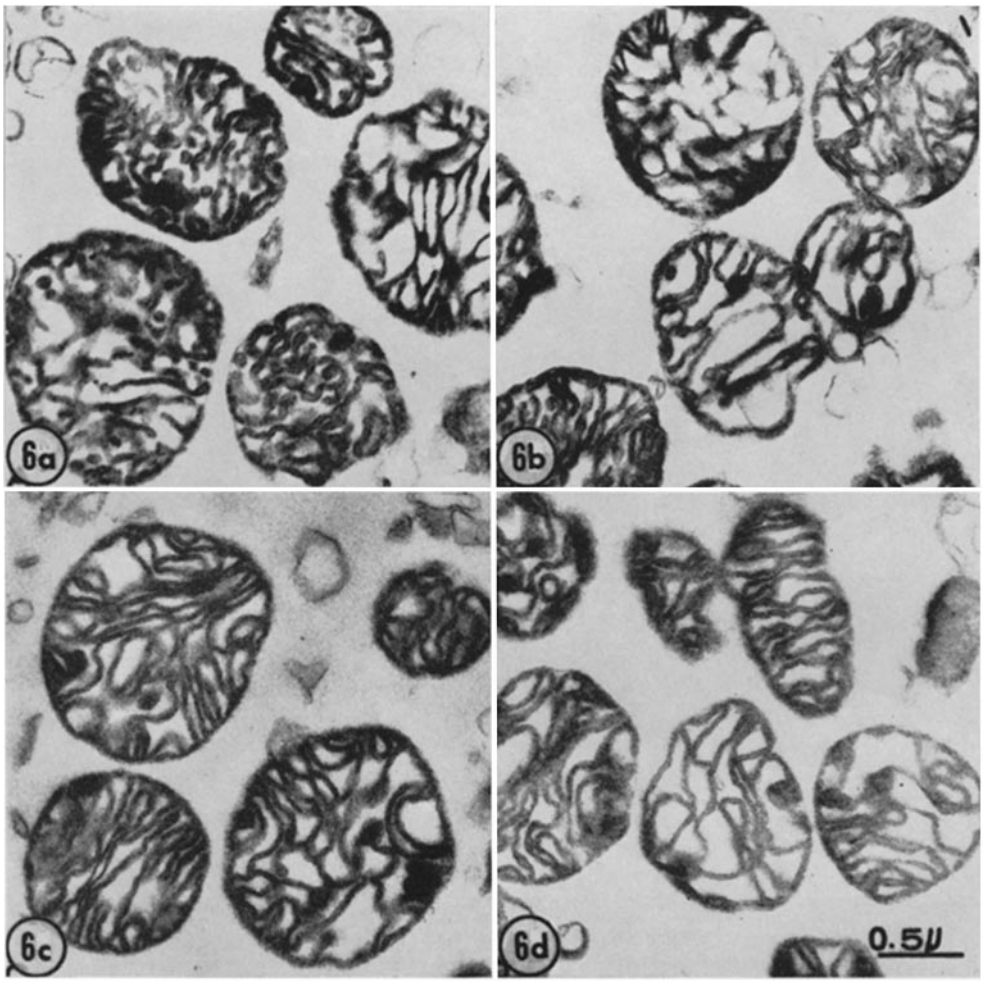

FIGURE 6 Mitochondria entering anaerobic conditions in the presence of substrate, P_i and ATP at 30°C. Respiration was initiated under state III conditions with ADP (0.25 mM) and allowed to enter state IV respiration for 2 min before becoming anaerobic. Other conditions included 4 ml reaction medium with 1 mg protein/ml. (*a*) aerobic 1 min; (*b*) anaerobic 30 sec; (*c*) anaerobic 1 min; (*d*) anaerobic 2 min. × 22,000.

cause the steady-state turnover of energy-linked reactions is minimal or nonexistent.

Figs. 5 and 6 show the effect of ADP and ATP on mitochondrial ultrastructure under anaerobic conditions. In addition, they examine a requirement of P_i-induced distention of inmerices. The aerobic controls have the familiar distended configuration in the presence of P_i (Fig. 5 *a*) and P_i plus ATP (Fig. 6 *a*). Upon entering the anaerobic state, the distended configuration is transformed into the condensed configuration although a few distended and coalesced forms are observed (Figs. 5 *b*, *c*, *d* and 6 *b*, *c*, and *d*). These results support the light-scattering data (11) from which it was postulated that mitochondrial compartments become condensed upon entering anaerobiosis. The addition of ADP to mitochondria under anaerobic conditions leads to further condensation of the inmerices, i.e., to coalesced and aggregated membrane configurations (Fig. 5 *e*). Fig. 6 shows ATP to be less effective than ADP in coalescing mitochondrial inner membranes under anaerobic conditions. ATP cannot condense inmerices with P_i present, and ATP does not contribute to P_i trans-

port under anaerobic conditions in order to maintain the distended configuration if this configuration is caused by P_i transport.

Osmotic Swelling and ADP

In the preceding sections of this report, changes in what has previously been called low amplitude swelling and contraction (42) were examined. The experiment described in Fig. 7 involves two general types of swelling-contraction phenomena. The first is large amplitude swelling brought about by passive osmotic manipulation as first quantitated by Tedeschi and Harris (43). This phenomenon has been studied recently with the electron microscope by Stoner and Sirak (44). The second volume change is a low amplitude contraction brought about by addition of ADP. Figs. 7 *a* and *c* show the distended inmerices of mitochondria generated by suspension in hypotonic sucrose solutions (12 mM and 62 mM, respectively) and fixed in isotonic glutaraldehyde fixative. Despite the absence of P_i, distended inmerices are observable. However, upon addition of ADP the dramatic ultrastructural rearrangement occurs, yielding mitochondria with coalesced and condensed inmerices. This is observed even with inmerices which have been extruded from lysed mitochondria (compare Figs. 7 *a* and *b*). Similar effects have also been observed in the presence of low levels of ADP (to 10 nmoles/mg protein), confirming the earlier observations of Packer (13). In this experiment, we have demonstrated an ultrastructural transformation (distended→condensed + coalesced) in the absence of energy transduction while oxygen consumption is decreased as seen in Table I. This transformation is similar to that originally proposed by Green and associates (8–11) to represent energy transduction as seen at the gross morphological level. Here we have the same transformation (compare Figs. 7 *c* and *d*) brought about by simple osmotic swelling followed by the interaction of ADP and mitochondrial inmerices.

Coalescence and Distention: An Examination of Mitochondrial Ultrastructure

Fig. 8 compares the morphology of mitochondrial inmerices in the presence of ADP (Fig. 8 *a*) and P_i (Fig. 8 *b*). Although these micrographs are of sufficiently high resolution and magnification to observe an 80 A headpiece, most particles range in diameter from 30 to 60 A (see arrows). The dimensions of the tripartite repeating unit (45) observed by the negative staining technique (headpiece, 80 A; stalk, 50 A; basepiece, 40 A) are difficult to confine to the 125 A thick, double membrane structures that are visible in Fig. 8 *a*. If the directional orientation is preserved with headpieces extending toward the matrix space as originally defined by Green (46), then a tremendous conformational change in the tripartite units opposing each other in the matrix had to occur in the presence of ADP. This idea has been advanced by Green and Baum (47). They diagrammatically show a very compact, tripartite repeating unit which represents the conformation of the structure under coalescing conditions. It must be pointed out that evidence for an extension-compaction cycle of the repeating unit is scanty and has not been demonstrated. However, it must be noted that reports have appeared which demonstrate the deleterious effects of chemical fixatives like osmium tetroxide and potassium permanganate on the tripartite repeating units (48, 49). Another interpretation to the very thin, double membrane areas may be that directional assignment of headpieces protruding into the matrix may not be a correct interpretation (Fig. 8 *a*). A recent paper by Heidrich (50), however, demonstrates a matrix side orientation for the headpiece of the inner membrane. Fig. 8 *a* also demonstrates the aggregation phenomenon which is often observed upon addition of ADP. There are several places (arrows only) where small globular structures are projecting from the inner membrane

TABLE I

Oxidation Rates of Mitochondria in Hypotonic Sucrose

Additions*	Sucrose concentration	mμ atoms oxygen/min per mg protein
	mM	
None	12	31.5
None	62	31.2
ADP	12	26.9
ADP	62	25.5

Pyruvate (5 mM) and malate (0.5 mM) were present in all reactions. 2 mg of mitochondrial protein were used in the 2 ml reaction suspension. ADP when present was added at an initial concentration of 0.5 mM. Reactions were run at 30°C.

* ADP was added to the mitochondrial suspension 2 min after reaction had begun.

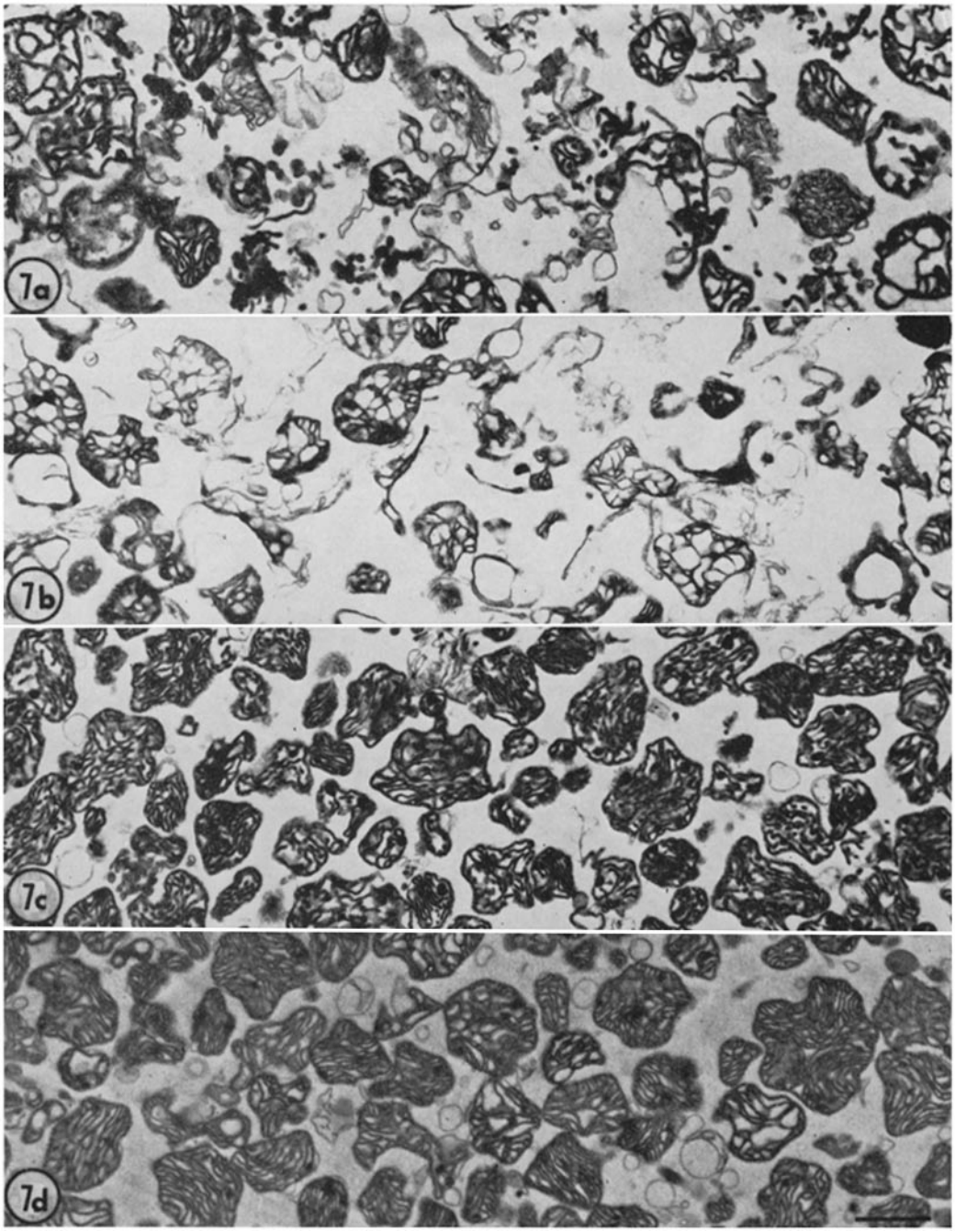

FIGURE 7 The effect of ADP on mitochondria under hypotonic conditions. Mitochondria were incubated in hypotonic sucrose solution (concentration given) in the presence of pyruvate (5 mM) and malate (0.5 mM) for 2 min at 30°C followed by ADP (0.5 mM) for 1 min where indicated. Samples were fixed with isotonic glutaraldehyde fixative at the conclusion of specified times. (*a*) sucrose (12 mM); (*b*) sucrose (12 mM) then ADP; (*c*) sucrose (62 mM); (*d*) sucrose (62 mM) then ADP. × 11,000.

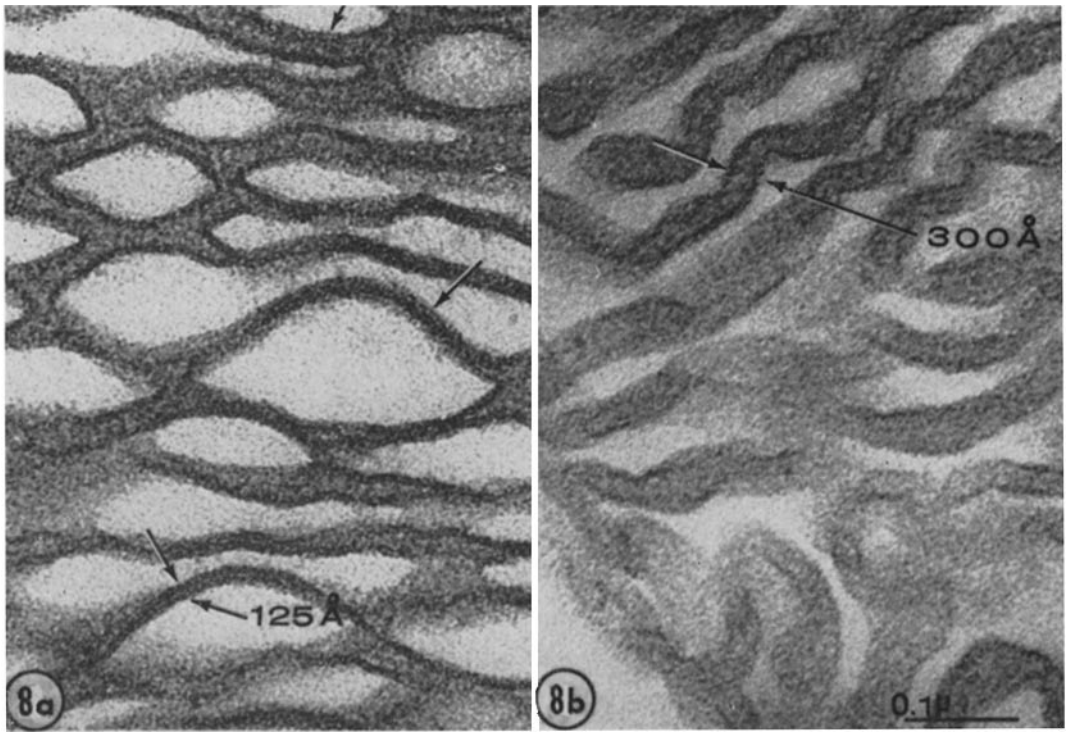

FIGURE 8 High magnification comparison of the effects of ADP and P_i on mitochondrial morphology. (*a*) Coalesced and aggregated configuration generated by substrate and ADP. (*b*) Distended configuration generated by substrate and P_i. × 150,000.

into the intracristal space. These structures average about 50 A in diameter and are considerably smaller than the 80 A headpieces observed in negatively stained preparations. In comparison, the thickness of inmerices is over 300 A at many places in the presence of P_i (Fig. 8 *b*). In addition, there are distended areas which measure 1200 A or more under the same conditions. It has been impossible to demonstrate 125 A inmerices in the presence of P_i. A final observation is that in the presence of ADP the inner mitochondrial membrane is 60–70 A thick at most coordinates and has a unit membrane appearance which is not apparent in the presence of P_i. This, however, may be due to somewhat poorer resolution (Fig. 8 *b*).

DISCUSSION

The new nomenclature introduced previously (29) and in the present report is an attempt to give not only a morphological terminology for mitochondrial ultrastructure but more importantly to stress the intimacy of the inner membrane-matrix relationship. This relationship has also been identified by other investigators (6, 51–53). Conversely, the concept of the crista is now viewed with less importance with its inner membrane-intracristal space relationship. The intracristal space is viewed as a series of channels allowing the substrates of metabolism essentially free and rapid access to the large surface area of the inner membrane. The reverse process facilitates the rapid diffusion of metabolic products from the inner membrane to the cytosol.

The intimacy with which ultrastructural changes are coupled to electron transfer, energy-linked processes or binding, and translocation phenomena has only been investigated in depth for a single class of compounds: the nucleotides. Of these, only ADP and dADP give rise to significant changes in ultrastructure. These effects have been traced to adenine nucleotide binding and translocation as shown in the experiment employing oligomycin and atractyloside (Fig. 4). The results, along with those employing ADP

at 0°C and 30°C, under hypotonic and anaerobic conditions, suggest that configurations of mitochondria in state III respiration reflect binding or translocation of ADP rather than a morphological expression of oxidative phosphorylation.

In pursuing the original claim that the configurational change observed in state IV and state III respiration is brought about by oxidative phosphorylation, Hackenbrock and coworkers (53) have demonstrated mitochondrial configurational changes in Ehrlich ascites tumor cell preparations. They were able to stimulate oxidative phosphorylation by increasing the ADP to ATP ratio in these cells through the use of 2-deoxyglucose. I interpret the associated ultrastructural change to be due to the increased level of ADP rather than a stimulation of oxidative phosphorylation. This point is substantiated by an earlier report by Packer and Golder (54) which mentions that a light-scattering increase was observed upon addition of glucose to anaerobic Ehrlich ascites tumor cells. The recent paper by Hackenbrock and associates (53) further demonstrates another point. Their experiment using oligomycin, an inhibitor of oxidative phosphorylation, shows condensation of mitochondrial inmerices when ADP was generated by the addition of 2-deoxyglucose. Oligomycin prevented stimulation of respiration by the hexose. Therefore the assumption that the condensed morphology observed with ADP and oligomycin reflects the energy-conserving mechanism of oxidative phosphorylation may not be warranted.

Hatase et al. (52) have now shifted their original position on ultrastructural changes and oxidative phosphorylation. These authors now believe that gross changes in mitochondrial morphology are correlated to "extensive conformational changes in matrix proteins." They maintain that configurational changes, signaled by conformational changes in matrix protein, really reflect primary conformational events due to electron transfer and oxidative phosphorylation. While this idea is certainly novel, it has at the present time no substantial experimental basis. Their report substantiates our previous finding (29) as well as those developed in the present communication. In essence, they too have found that distention of mitochondrial inmerices requires high levels of P_i (1 mM) along with electron transport. However, they observe P_i distention of inmerices at 1°C whereas we were unable to generate the distended configuration at 0°C. We are only able to observe mitochondria in the distended configuration at 0°C by first incubating them at some higher temperature with P_i and substrate and then rapidly cooling them to 0°C. This observation superficially appears to conflict in part with the results of the anaerobic experiment. First, it says that at 0°C, P_i does not distend inmerices due to lack of electron transfer. This result is shared by both experiments. However, the results of the anaerobic experiment dictate that the distended configuration must collapse in the absence of energy required to maintain the internal osmotic pressure. The fact that distended mitochondrial inmerices rapidly cooled to 0°C do not collapse immediately is probably due to temperature. The decreased mobility of the membrane lipid components as well as ion activity may account for part of this observation.

The results of the hypotonic experiments strongly indicate that distended inmerices are merely a reflection of increased matrix volume brought about by the active or passive accumulation of ions and water. All processes studied appear to utilize primarily the energy of electron transfer for swelling-contraction phenomena except for passive osmotic changes and low amplitude contraction brought about by ADP or dADP. The fact that the latter effect is demonstrated with an apparent lack of energy source brings one to the conclusion that the primary event of binding, exchange, or translocation of these nucleotides triggers a conformational event within the inner mitochondrial membrane. This presumably initiates a mechanism by which ions and water are extruded from the matrix. Speculation at this point could include a Donnan type effect as previously discussed by Young et al. (55).

In summary, this report extends previous findings concerning adenine nucleotides and configurational changes to include the coalescence of mitochondrial inmerices under anaerobic and hypotonic conditions. It delineates the effects of adenine nucleotide diphosphates versus triphosphates, the former showing condensation of inmerices whereas the latter do not in the presence of oligomycin. The relationship between phosphate distention and electron transport was affirmed. In addition, the development of a new morphological terminology based on the cooperative and intimate relationship between the inner mitochondrial membrane and the matrix is

suggested for mitochondrial ultrastructure. Finally, our results indicate that ADP binding or translocation is the basis of the configurational changes observed and is distinct from oxidative phosphorylation. Although there is little doubt that protein conformational changes at the molecular level do occur, gross changes seen at the level of the electron microscope probably reflect osmotic changes.

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