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STUDIES ON THE STRUCTURE AND FUNCTION OF BIOLOGICAL MEMBRANE SYSTEM*

II. CONFIGURATIONAL CHANGES OF MITOCHONDRIA *IN SITU* CORRELATING TO FUNCTIONAL STATES

YOHEI TAKAHASHI

Department of Pathology, Nagoya University School of Medicine
*(Director: Prof. Masasumi Miyakawa)***

ABSTRACT

Configurational changes of the mitochondrial inner membrane system were examined ultrastructurally and biochemically. Mitochondria in intact heart muscle appeared predominantly in a so-called "orthodox" mode. On the other hand, the mitochondria isolated from liver or heart muscle showed a so-called "aggregated" mode.

Many authors who have examined extensively isolated mitochondria found that they underwent ultrastructural changes coupled with their functional metabolic states, corresdonding to the surrounding conditions such as substrates, Pi, nucleotides or uncouplers.

Intact heart muscles were fixed by perfusion with glutaraldehyde solutions, and two types of configurational states of mitochondria were found. The first type of mitochondria showed an irregular and relatively straight arrangement of the inner membrane system. The second had an angular and regular configuration. Ehrlich ascites tumor cells also exhibited the twisting and waving configuration of mitochondrial inner membrane when fixed at the state of respiratory inhibition induced by addition of glucose. Mitochondria of fasted ascites cells showed an irregular configuration.

Mitochondria in the rough homogenates of rat heart muscle had morphologically a transitional structure between isolated mitochondria and ***in situ*** ones. Mitochondria in the homogenates fixed at various functional metabolic states showed particular configurational changes correlating to their functional states in both "orthodox" and "aggregated" modas.

From ***in vitro*** experiments, the angular or twisted configuration of the inner membrane of mitochondria was found to be induced by oxidizable substrates and Pi. As intact cells possess Pi endogenously, the mitochondria in situ are considered to have two different configurations.

高 橋 洋 平

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* Abbreviations used: Pi, inorganic phosphate; DNP, 2, 4-dinitrophenol; EDTA, ethylenediamine tetraacetate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate.

** Retired from Nagoya University School of Medicine March 31, 1969.

INTRODUCTION

In recent years, with the progress of electron microscopic investigations, much information on the detailed structure of mitochondria in intact cells has become available[1)2)3)4)5)]. It is well known that there are remarkable variations in the size, shape and internal structure of mitochondria in different kinds of cells[6)], and that the mitochondrial membrane system serves not only as structural envelope but also contains the highly organized assemblies of respiratory enzymes which are responsible for formation of ATP, for active transport and for the mechanochemical energy coupling involved in shape and volume changes[7)8)9)].

Isolated mitochondria from rat liver or beef heart have been extensively studied and shown to undergo ultrastructural changes under a variety of conditions[10)11)12)13)14)15)16)17)18)19)]. From a number of reports, it is evident that the ultrastructural changes in mitochondria are correlated with the functional metabolic state in the mitochondrion.

Based on a number of evidences of systematic ultrastructural changes in isolated mitochondria under various respiratory states, Green and his co-workers proposed that the reversible transformation of utilizable energy in the mitochondiron is released by an oxidative-reductive reaction into conformational energy[20)21)]. Configurational changes in the inner membrane system are assumed to be reflections of corresponding conformational changes in electron transfer chain[22)].

On the other hand, mitochondria exhibit various ultrastructural changes involving swelling, contraction and degeneration under different sorts of physiological and pathological conditions in the living cells. It is very interesting and fascinating to find what kind of configurational changes can correspond to respiratory metabolic states in mitochondria *in situ*. There are few papers which have demonstrated configurational changes in mitochondria *in situ* under controlled experimental conditions[23)]. However, it has not been adequate to establish the identification of the changes of the inner membrane system in cross relation to functional states of mitochondria *in situ*. On the other hand, mitochondria in intact tissues are observed predominantly in a so-called "orthodox" mode. It is difficult for mitochondria in intact tissues to stay under a desired particular experimental condition because they are protected by their cytoplasmic environment. Conventional fixation methods now available for electron microscopy are unsatisfactory for complete fixation of mitochondria in intact tissues in the state that they are.

In consideration of these views, the author studied the configurational changes of mitochondria *in situ* and attempted to find the essential changes of the mitochondrial inner membrane under various states of respiration. For these purposes, in the present study: (i) mitochondria in Ehrlich ascites tumor cells, (ii) mitochondria in the rough homogenates of rat heart, (iii) isolated

rat heart mitochondria and (iv) mitochondria in the intact rat heart muscle were used as materials to demonstrate the correlation between the configurational changes and functional states of mitochondria *in situ*.

MATERIALS AND METHODS

Preparatian of Ehrlich Ascites Tumor Cells

Ehrlich ascites tumor cells were inoculated into 2–3 month-old Swiss mice and were withdrawn from the peritoneal cavities of the mice about eight days after inoculation. The cells were collected and washed several times with ice-cold phosphate buffered saline (PBS) by centrifuging in a clinical centrifuge at 800 rev. per min. for 5 minutes. Finally the cells were suspended in PBS at a concentration of approximately 5 millions of cell count per cubic millimeter. The protein concentration was 2 mg protein per 10^9 cells.

Preparation of Rat Heart Rough Homogenates

Adult albino rats were sacrificed by decapitation. The beating hearts were taken out and immediately placed in an ice-cold medium of 0.21 M mannitol, 0.07 M sucrose, 0.1 mM EDTA and 5 mM Tris-chloride, pH 7.4. After being minced, the heart muscle was gently homogenized in a loosely fitted Polytfon homogenizer type 20 ST. The homogenates were fractionated by differential centrifugation at 700 G for 10 minutes to remove free mitochondria.

Preparation of Rat Heart Mitochondria

Rat heart mitochondria were prepared from heart muscle following treatment with Nagarse proteinase by the method of Hatefi *et al.*[24] The final pellet of heavy mitochondria was suspended in a concentration of 50 mg of protein per ml in a solution which was the same as the medium employed for the preparation of the rough homogenates.

Measurement of Oxygen Uptake

Oxygen uptake by isolated mitochondria and rough homogenates was measured at 25°C with a Clark type oxygen electrode (Beckman Co., Ltd.). The experiments were carried out in a medium (5 ml) containing 0.3 M mannitol, 10 mM KCl, 2.5 mM $MgCl_2$ and 0.25 mM EDTA, at pH 7.4 adjusted with KOH. Sodium succinate (8 mM) was used as an oxidizable substrate. The uncoupler was DNP (20 μM), unless otherwise stated. The concentration of protein in mitochondria and rough homogenates was 2 mg per ml of the reaction medium respectively.

Respiration of Ehrlich ascites tumor cells at the concentration of 20 mg protein per ml was also measured polarographically in 2.5 ml of PBS at 25°C.

Determination of Phosphorus Content

Inorganic phosphate and total phosphorus content were analysed on heart

muscle, liver tissue, Ehrlich ascites tumor cells and liver mitochondria. Blotted fresh tissues were weighed and their specific gravities were measured by the volume changes of the media in which they were soaked. Samples were then homogenized by a homogenizer in the same mannitol medium as used to prepare rough homogenates and mitochondria. On these homogenate, ascites tumor cells and mitochondria, inorganic phosphate was measured by the method of Chen *et al.*[25] and of Berenblum and Chain as modified Martin and Doty. Total phosphorus was measured by the method of Chen *et al.*

Determination of Protein

The biuret method of Gornall *et al.*[26] was applied to all the samples solubilized by the addition of 0.05 ml of a 10% solution of potassium deoxycholate.

Electron Microscopy

(i) *Fixatives;* The fixative used in most experiments was 2% glutaraldehyde with 0.25 M sucrose and 0.05 M potassium cacodylate, pH 7.4. For special purposes, glutaraldehyde fixative which containd 0.15 M NaCl or 0.1 M phosphate buffer was used, instead of sucose and cacodylate. After fixation with glutaraldehyde, all the samples were post-fixed at 0–4°C in a solution of 1% osmium tetroxide, with 0.25 M sucrose and 0.05 M potassium cacodylate, pH 7.4.

(ii) *Perfusion Fixation of Rat Heart*; Adult albino rats were used. The left thorax was quickly opened and the left ventricle of the heart was injected with glutaraldehyde fixative by a syringe. The pressure of fixative was maintained so that the ventricle was slightly distended. By this perfusion the heart always stopped beating after two or three strokes. Then small blockes from the left ventriclar wall were cut and further fixed with the same fixative for one hour at room temperature and post-fixed with osmium tetroxide solution for two or three hours at 0–4°C.

(iii) *Procedure of Conventional Electron Microscopy*; The incubated, isolated mitochondria, rough homogenates of heart muscle and ascites tumor cells were fixed by quick mixing with fixative when they reached a particular energy state monitored by the oxygen electrode. The final concentration of glutaraldehyde was 1%. These mixtures were allowed to stand for 15–30 minutes at room temperature. The mitochondria and ascites cells were centrifuged at 3,000 to 10,000 G to form pellets. The pellets were cut into small blocks which were about one cubic millimeter in size. The rough homogenate were collected by a clinical centrifuge. After washing with the same buffer, they were post-fixed with 1% osmium tetroxde in the same buffer for 1–2 hours at 0–4°C.

All samples were dehydrated in graded series of ethanol and embedded in Epon[27]. The thin sections were cut by glass knives with a Sorvall Porter-Blum MT-2 ultramicrotome. The sections were mounted on carbon covered grids, stained with uranyl acetate and lead citrate[28], and examined by a Hitachi HU-

11 D electron microscope.

RESULT

Responses of Respiratory Metabolism

Polarographic measurement of respiration in Ehrlich ascites tumor cells revealed that their respiratory responses to the exogenous substances were quite different from those of heart muscle homogenates or of isolated mitochondria.

Oxygen uptake by ascites tumor cells depends on the condition of the cells. As illustrated in Fig. 1, the slow rate of endogenous respiration could not be activated by the addition of any exogenous substrate after treatment of preincubation in the presence of glucose (6 mM). When the cells were incubated in glucose free medium for 4 hours at 0–4°C, the slow rate of endogenous respiration (7.5 mμ atoms 0/min./mg protein) could be rapidly accelerated by addition of glucose (30 mμ atoms 0/min./mg protein). However, addition of succinate (8–16 mM) or of pyruvate (2 mM) and malate (0.2 mM) did not show any effect on respiration of ascites cells. This respiratory activation continued for about 1 minute and was regularly followed by in-

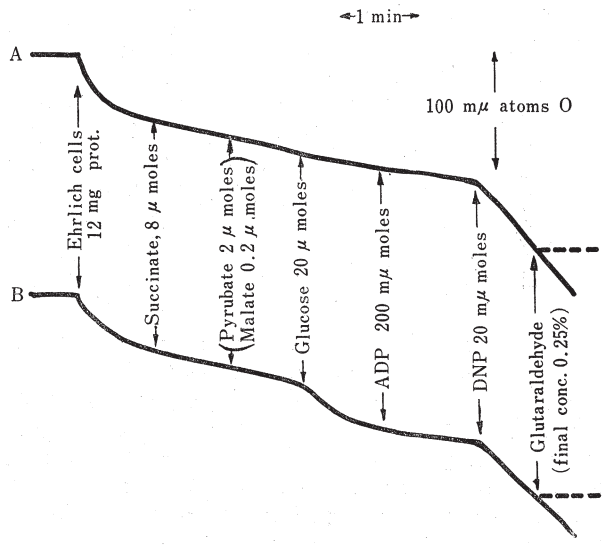

FIG. 1. Oxygen uptake by ascites tumor cells

The reaction medium was PBS, pH 7.3; The total volume was 2.5 ml; the temperature, 25°C; it contained 12 mg protein of Ehrlich cells per ml; the amounts are given as per ml. Preincubated cells in the presence of glucose (A) could not be activated by any exogenous substrates. Fasted cells incubated in a glucose free medium (B) were rapidly activated by addition of glucose. DNP released the inhibitory respiration. Effects of glutaraldehyde were shown by dashed lines.

hibition as mentioned by Chance and Hess[29]. The state of inhibition could be relieved by adding DNP[30]. Neither added ADP nor calcium chloride (0.2–2 mM) had any effect on the respiration.

Oxygen uptake by isolated mitochondria was approximately 5 times as much as that by the ascites cells. The respiration of the isolated mitochondria was characterized by very clear responses to the oxidizable substrates, ADP and uncouplers (Fig. 2 A). In comparison with isolated mitochondria, the rough homogenates of heart muscle showed lower respiratory control and slower responses to oxidizable substrates and ADP. However, they had more respiratory activity than the intact cells such as ascites cells (Fig. 2 B).

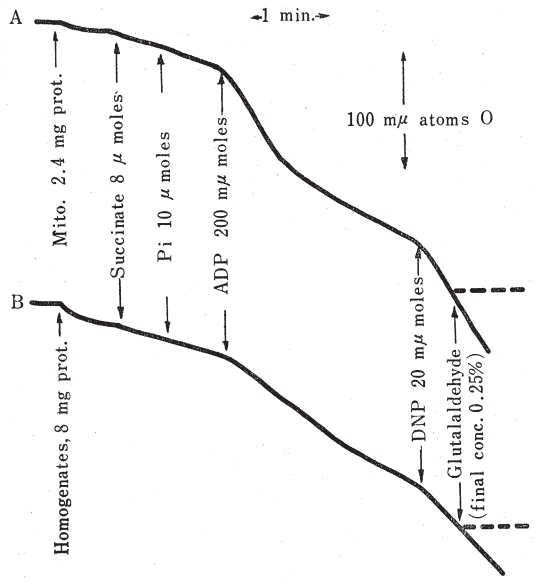

FIG. 2. Oxygen uptake by isolated rat heart mitochondria (A) and rough homogenates (B)

The reaction medium was 0.3 M mannitol, 10 mM KCl, 2.5 mM $MgCl_2$ and 0.25 mM EDTA, pH 7.4, The total volume was 5 ml; the temperature, 25°C; it contained 2 mg and 8 mg protein of mitochondria and homogenates repectively; the amounts are given as per ml. Though the homogenates showed lower respiratory control and slower responses than isolated mitochondria, they showed essentially the same character.

In order to examine the effect of glutaraldehyde fixative on respiration in each preparation, glutaraldehyde was added into the reaction medium at various respiratory states. It was confirmed that glutaraldehyde at final concentrations of 0.25 to 0.5 per cent could sufficiently stop their respiration within several seconds at all times (Fig. 1 and 2).

Electron Microscopic Observations

Intact rat heart tissues were fixed initially by perfusion with various glutaraldehyde solutious of different compositions, such as: (i) 2% glutaraldehyde in 0.25 M sucrose and 0.05 M K-cacodylate, pH 7.4, (ii) 2% glutaraldehyde in 0.15 M NaCl, 5 mM Tris-Cl, pH 7.4, and (iii) 2% glutaraldehyde in 0.1 M phosphate, pH 7.4. Almost all mitochondria in the intact tissue were in "orthodox" mode. Mitochondria fixed with glutaraldehyde in sucrose and K-cacodylate showed moderately dense matrix, straight and parallel cristae with many fenestrations (Fig. 3 A). The zigzag configuration of cristae was occasionally observed in mitochondria fixed with glutaraldehyde in NaCl or in phosphate buffer (Fig. 3 B).

Two types of mitochondria *in situ* could be observed clearly by the configurational change in the inner membrane system. The first type was commonly seen in intact cells of tissues fixed by the conventional method for electron microscopy, and the second one was demonstrated under certain special conditions. Both types of mitochondria showed the structural characteristics of so-called "orthodox" mode such as tubular cristae, small intracristal space and large matrix space, in sharp contrast to isolated mitochondria. In the first type, the inner membrane system of mitochondria was characterized by irregular running lines; nevertheless it was oriented in relatively parallel arrays like the form in cristae (Fig. 3 A). In the second type, the inner membrane system appeared remarkably in angular configuration consisting of regularly repeating concavity turned toward the matrix, and frequently forming zigzag cristae (Fig. 3 B).

Mitochondria in ascites tumor cells under fasted condition were employed to find out the relationship between configurational changes and functional states of the mitochondrial inner membrane. When the cells were successfully fixed, there was marked evidence that in a large portion of mitochondria the membranes of the cristae greatly changed their configuration of the state of respiratory inhibition after glucose addition to the cells. The membrane formed a continuous wave-like curve concaving toward the matrix in the sections where the cristae were sectioned along the long axis. The closely packed cristae appeared in a reticulate pattern in areas where the cristae were sectioned perpendicularly to their long axis (Fig. 4 A). The same configurational change of the inner membrane system in the cells as described above could also be observed in the non-fasted cells incubated in the medium which was saturated with oxygen by continuous bubbling, although the population of this change in the mitochondria was not so great.

When ascites tumor cells were fixed at the released state of respiration induced by DNP addition in the presence of glucose, the membrane of the cristae showed a configuration with no regularity in their running and arrangement (Fig. 4 B). There were usually observed moderately condensed

matrix and relatively large intarcristal space in the mitochondria. Most of them at this state of respiration belonged to the first type of mitochondria as mentioned above. When an oxidizable substrate such as succinate was added to the incubation medium in the absence of glucose, the membrane of the cristae demonstrated almost the same configurational change as that in the mitochondria at released state (Fig. 4 C). There were exceptionally seen mitochondria in the second type. It is safe to say that exogenous oxidizable substrate such as succinate or pyruvate and malate had no effect on the configurational change in the inner membrane system.

In general, the structure of isolated mitochondria was quite different from that of mitochondria *in situ* and showed a so-called "aggregated" mode[20] characterized by large intracristal space, small matrix space and long distance from outer to inner membrane. When succinate (8 mM) was added as oxidizable substrate to the reaction medium, mitochondria showed relatively straight and parallel arrangement of the inner membrane. The matrix space was slightly expanded and was 250 to 300 Å in thickness. There were occasionally seen ladder-like anastomoses of the matrix. In the presence of Pi (10 mM), most of the inner membrane systems showed a characteristic configurational change which consisted of concavity toward the matrix and twisting as a whole. Thus, the matrix space was enveloped by the twisted tube of the inner membrane system (Fig. 5 C). The diameter of the tubular cristae was approximately 300 to 500 Å. In some mitochondria the cristae formed a zigzag mode of parallel arrangement. After addition of DNP (20 μM), the inner membrane system became flat with an irregular net-work of sheet-like matrix which was 150 to 250 Å in width (Fig. 5 A). The intracristal space occupied a major portion of the whole space in mitochondria. This configuration at a released state of respiration was characterized by an irregular arrangement of the inner membrane system.

For the explanation of the configurational changes in mitochondria *in situ* relating with those in isolated mitochondria, an experiment was performed on rough homogenates which contained transitional forms of mitochondria between isolated mitochondria and *in situ* ones. In rough homogenates of rat heart muscle, there appeared usually two modes of mitochondria according to the mechanical force given during homogenization. When intensely homogenized, most of the mitochondria showed a resemblance to isolated mitochondria in so-called "aggregated" mode. They showed large intracristal space and small space of electron dense matrix (Fig. 6 A and B). On the other hand, as a result of less intense homogenization, a large portion of the mitochondria was in a so-called "orthodox" mode composed of small intracristal space and an electron transparent matrix. They were similar to mitochondria *in situ* (Fig. 6 C and D). Rough homogenates of rat heart were suspended (8 mg protein per ml) in the same reaction medium. In the presence of Pi and succinate,

there was demonstrated successfully a particular configuration of the inner membrane system of mitochondria in both modes (Fig. 6 B and D). They showed essentially the same configurational change which was characterized by regular twisting and concaving of the inner membrane system toward the matrix. The difference in their structure was only found in the volumes of the matrix in "aggregated" and "orthodox" modes. Addition of DNP could induce mitochondria to the released state of their respiration and the inner membrane system became flat in both modes (Fig. 6 A and C), so the cristae became irregular in shape. The volumes of the matrix spaces were also different between both modalities.

Measurement of Inorganic Phosphorus in Living Cells

It was found that the quantitative distribution of Pi in ascites tumor cells was very similar to that in either liver cells or heart muscle. As shown in Table I, there was contained Pi at the concentration of approximately 10 mM in the intact tissues. Addition of Pi at the minimum concentration of 0.5 mM to the reaction medium was needed for the particular twisted configurational change of the inner membrane system at a high energy state of respiration in isolated mitochondria[31]. Although the data were limited, it could be concluded that the concentration of Pi in the living cells was sufficient to make the inner membrane system of mitochondria take the particular configuration. Therefore, it can be considered that in mitochondria *in situ* there are only two configurational states which are distinguished by twisting or non-twisting of the inner membrane.

TABLE 1. Content of phosphorus

Materials	Inorganic phosphate		Total phosphorus
	μ moles/mg protein	mM in wet tissue	μ moles/mg protein
Rat Heart muscle	0.045	11.6	0.236
Liver tissue	0.044	10.0	0.208
Liver mitochondria	0.017	—	0.265
Ehrlich ascites tumor cells	0.053	—	0.457

DISCUSSION

In the present study the configurational changes of mitochondria *in situ* correlating to functional states were observed. There are some difficulties in the demonstration of the configurational changes, such as fixation problem and controlling condition of functional state of mitochondria in the living cells. As the speed of the configurational changes of mitochondria is compatible with the turnovers of the known components[22], the fixatives should reach the mitochondria through the cell membrane and other intracellular components and react on

them much faster than they should change their configuration. Therefore, the natural figure of mitochondria *in situ* may be recognizable only by chance[32)33)]. Furthermore, it is difficult to let mitochondria be in intact tissue under a desirable particular metabolic state with some exceptional cases.

To clarify the relation between the configurational changes and the energy metabolism, Ehrlich ascites tumor cells and rough homogenates of rat heart were chosen. Respiratory states of these materials were monitored by oxygen electrode so that fixation was exactly done at the moment when they were in particular metabolic states. As the subsequent deceleration of Ehrlich cells' respiration following glucose addition is attributable to the increase of ATP and to the decrease of the concentration of ADP in the mitochondria[29)], the twisting configuration of this state was considered to have some connection with the high energy state of the inner membrane system.

A similar phenomenon was reported by Saladino *et al.*[34)] They demonstrated condensed and twisted mitochondria of toad bladder urinary epithelium which was treated by the polyene antibiotic Amphotericin B. They postulated that Amphotericin B caused the cell volume expansion as a result of the influx of sodium and water, and the condensation of mitochondrial matrix was a metabolically-linked phenomenon related to the rapid hydrolysis of ATP to ADP in cytoplasm by the sodium pump.

As the homogenates lack the intact plasma membrane, mitochondria in the homogenates are not *in situ* in a strict sense, but by their close structural similarity, they might be regarded as the transitional ones between isolated mitochondria and those *in situ*.

Green and co-workers[20)21)22)] have described three configurational states ("non-energized", "energized" and "energized-twisted") of the inner mitochondrial membrane system, and also mentioned these morphological changes to be correlated with the functional states in the mitochondrion. The "non-energized" configurations were induced by uncouplers, ADP, translocation of divalent metal ions, ion-induced swelling and the sbsence of oxidizable substrates or the presence of respiratory inhibitors. The "energized" configurations were generated by oxidizable substrates or ATP. When "energized" mitochondria were exposed to Pi (3–10 mM), mitochondria were immediately transformed into the "energized-twisted" configuration.

The configurational changes of isolated mitochondria presented by Green *et al.* have been confirmed in rat heart mitochondria.

It is well known that the mitochondrion is basically composed of two membrane systems (outer and inner membrane) and two spaces (intermembrane space bounded by the two membrane systems and matrix space enclosed by the inner membrane). But many interpretations are still being made on the inner membrane system. Penniston *et al.*[20)] distinguished the cristae from the inner boundary membrane. They postulated the former as tripartite and the

latter as monopartite.

Santiago *et al.*[35] proposed from their observation of negatively stained mitochondria the tubules covered by projecting subunits as branches inserted on the cristae. By their scheme, the cristae and the inner membrane opposite to the outer membrane completely lack projecting subunits.

These postulates, however, lack adequate experimental support. It is advisable at present to regard the inner membrane system as a single unit and any portion of it has the same conformation and the same possibility to change its configuration.

Harris *et al.*[21] presented also a determination of the inner membrane of mitochondria by the mode of the cristae ("orthodox", "aggregated" and "comminuted"). Many authors use such terms as "orthodox and condensed" or "expanded and contracted". Although, there are some differences in meaning, these terms are almost synonymous. Supposing that the terms "orthodox" and "aggregated" are representatives of the modalities, what is the essential difference between these two words? It is obvious that ultrastructurally the difference exists in relative volume change of the matrix space and intermembrane space. The "orthodox" mitochondrion shows little intermembrane space and much matrix space; however, the "aggregated" one shows large intermembrane space and decreased matrix space. And that is the case of mitochondria *in situ* and isolated ones. The most striking difference between isolated and *in situ* mitochondria is in the volume, especially in that of the intracristal space.

As described above, metabolically-linked configurational changes of the mitochondrial inner membrane were observed in isolated rat heart mitochondria, rat heart rough homogenates, intact rat heart muscle, occasionally, and Ehrlich ascites tumor cells. From these results it is proposed that mitochondria *in situ* take two basic configurations correlated to their functional states. The relation of this configurational change is semi-diagrammatically illustrated in Fig. 7. Provided oxidizable substrate and oxygen are supplied sufficiently, mitochondria are immediately changed into the twisting configuration inevitably by endogenous Pi. When oxidative phosphorylation occurs, mitochondria will be transformed into the non-twisting configuration. Transformation of the mitochondrial inner membrane between these two configurations will repeatedly continue as long as oxidative phosphorylation continues. The turnover rate may be regulated by many factors in the cell.

Although the thesis proposed by Green *et al.*[22] that the "energized" states of the inner membrane are the functional equivalents of the high energy intermediates could not be proved from these experiments, the existence of the configurational changes of the inner mitochondrial membrane *in situ* correlating to functional states was recognized.

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LEGENDS FOR FIGURES

FIG. 3. Mitochondria in rat heart muscle fixed by glutaraldehyde perfusion:
A, Fixed initially with standard solution which contained 2% glutaraldehyde, 0.25 M sucrose and 0.05 M potassium cacodylate, pH 7.4. Relatively straight and parallel arrangement of the inner membrane is seen. The left lower one was sectioned perpendicular to its axis.
B, Fixed with 2% glutaraldehyde solution containing 0.15 M NaCl. Angular configuration of the inner membrane is clearly seen. Magnification: ×60,000.

FIG. 4. Ehrlich ascites tumor cells:
A, Fixed at the state of respiratory inhibition after glucose addition (20 mM). Mitochondria which have twisting configuration of the inner membrane are seen. Concavity of the inner membrane toward matrix is characteristic pattern of this configuration. Magnifications: inset, ×44,000; large field, ×22,000.
B, Fixed at the released state of respiration induced by DNP in the presence of glucose. All of mitochondria showed the irregular configuration. Magnification: ×22,000.
C, Fasted ascites cell fixed after addition of succinate (8 mM) in the absence of glucose. Most of the mitochondrial inner membrane are irregularly arranged. Magnification: ×22,000.

FIG. 5. Isolated rat heart mitochondria in mannitol medium:
A, Fixed at the state of released respiration induced by DNP (20 μM) in the preseuce of succinate. The inner membrane system became flat and ittegular net-work of sheet-like matrix.
B, Fixed when succinate (8 mM) was added as substrate in the absence of Pi. Mitochondria showed relatively straight and parallel arrangement of the inner membrane.
C, Fixed at the state 4 respiration. Succinate (8 mM) and Pi (10 mM) were present. The inner membrane system showed characteristic configurational change. Concavity toward matrix and twisting as a whole are remarkable. Magnification: ×40,000.

FIG. 6. Mitochondria in rat heart rough homogenates:
As A and B were intensely homogenized materials, mitochondria are in so called "aggregated" mode. C and D were less intensely homogenized, mitochondria are in "orthodox" mode. A and C, Fixed at the released state of respiration induced by DNP in the presence of succinate and Pi. B and D, Fixed in the presence of succinate and Pi. The twisting or angular configuration of the inner membrane is observed in both modes. Magnification: ×40,000.

FIG. 7. Semi-diagrammatic representation of the proposed configurational changes of mitochondria:
A and B, Mitochondria *in situ* in "orthodox" mode. The angular configuration (A) is generated by intracellular oxidizable substrates or ATP, and transformed into the non-angular configuration which is induced by ADP, divalent metal ions, movalent salts or uncouplers.
C, D and E, Isolated mitochondria which are the same configurations as shown in Fig. 5 C, A and B, respectively.
A corresponds to C, and B corresponds to D. The state of E is not exist theoretically in mitochondria *in situ*.

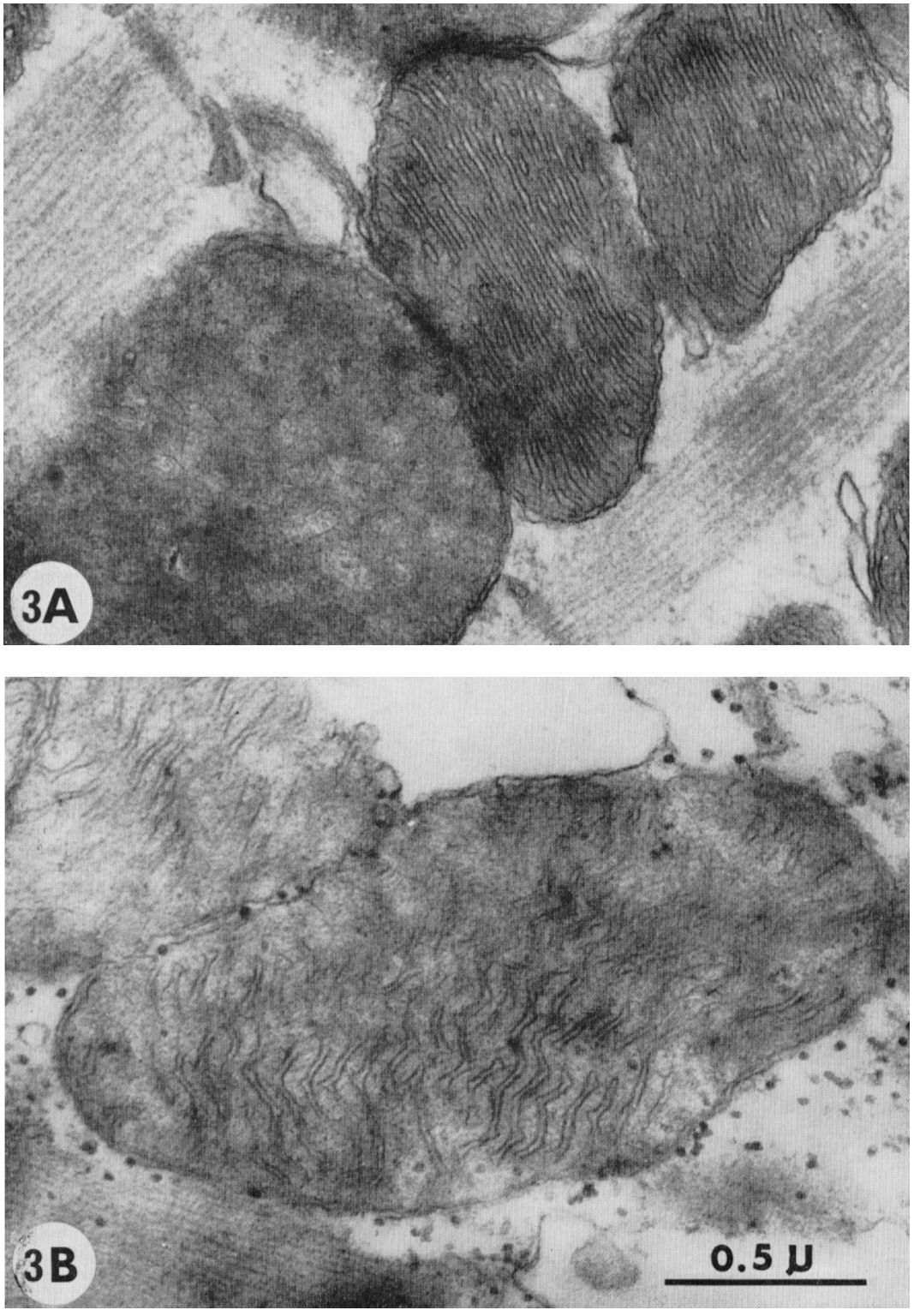
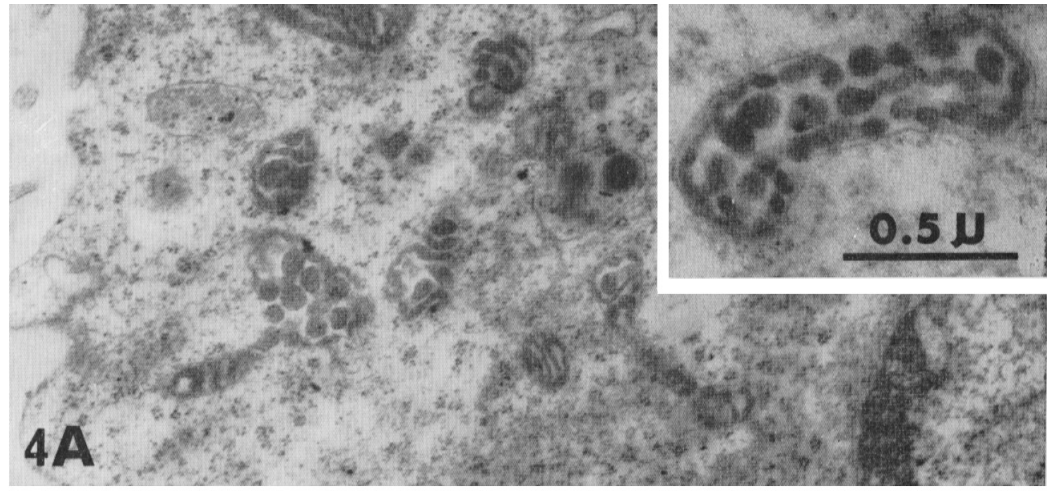
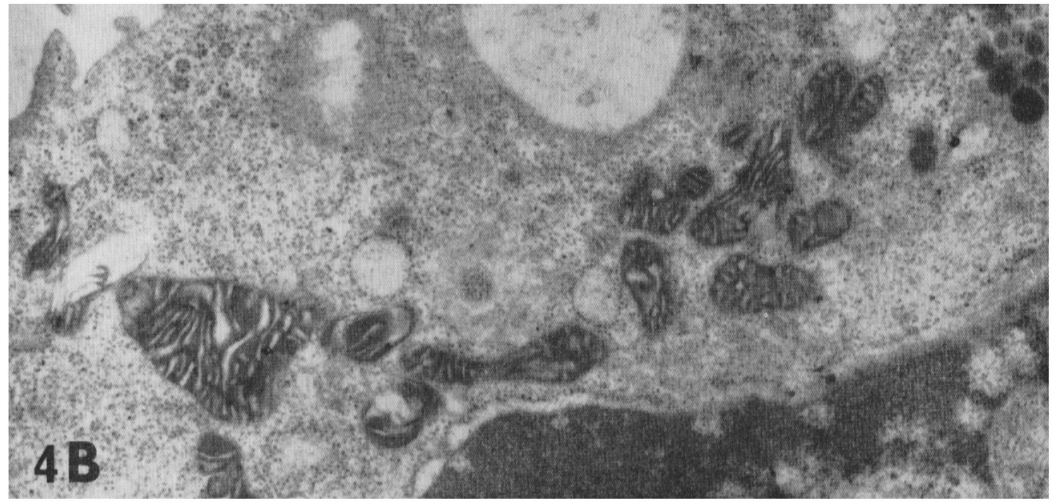


FIG. 3





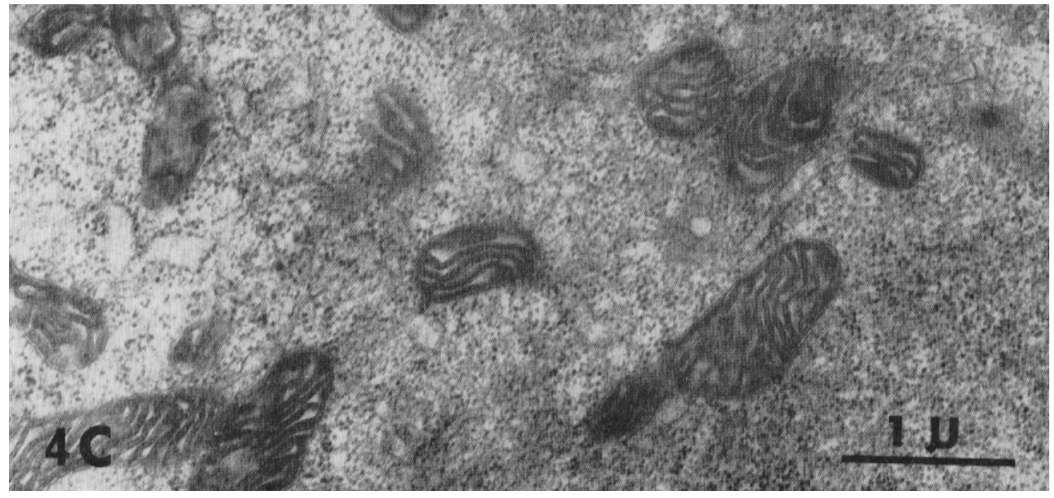


FIG. 4

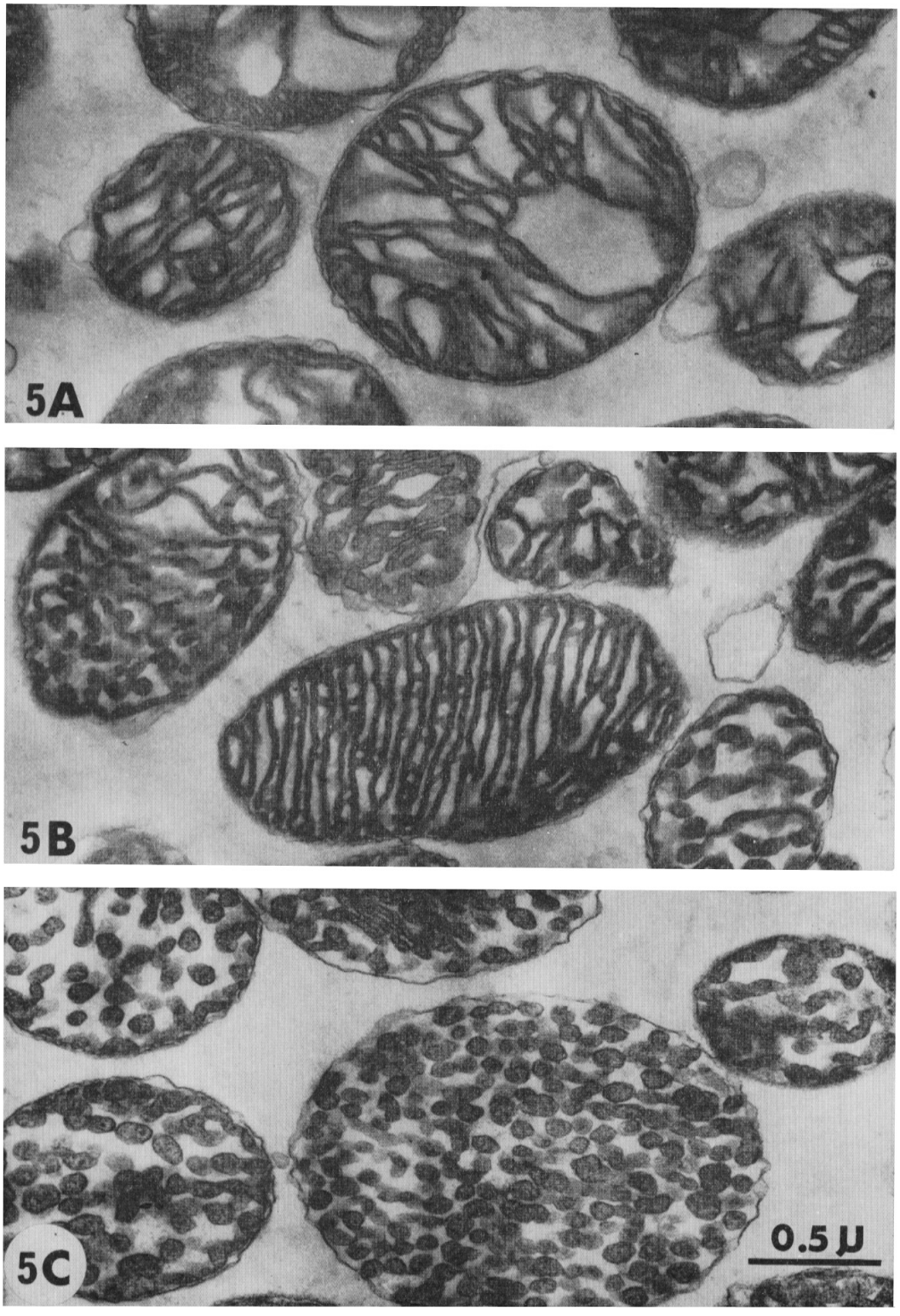


FIG. 5

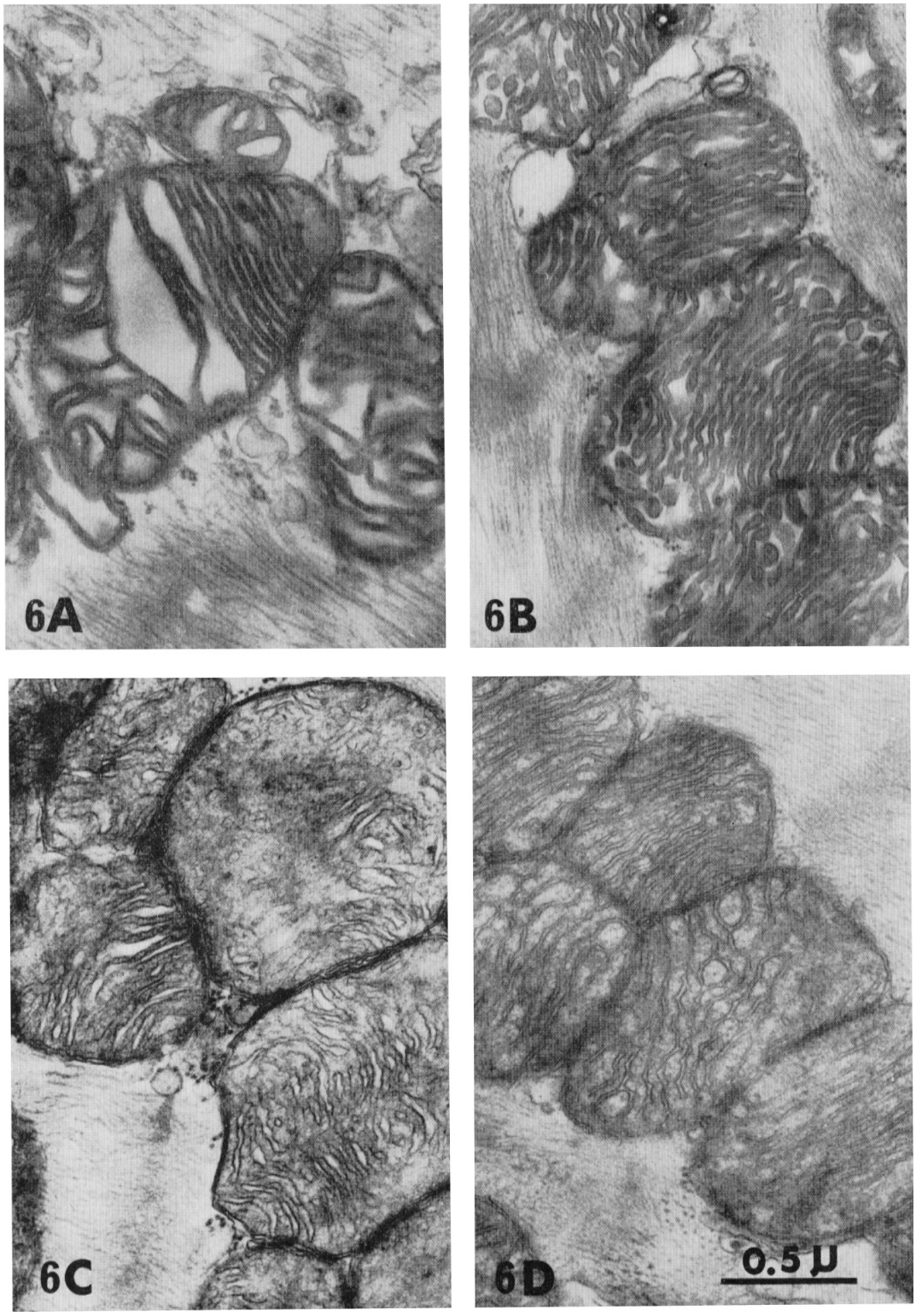


FIG. 6

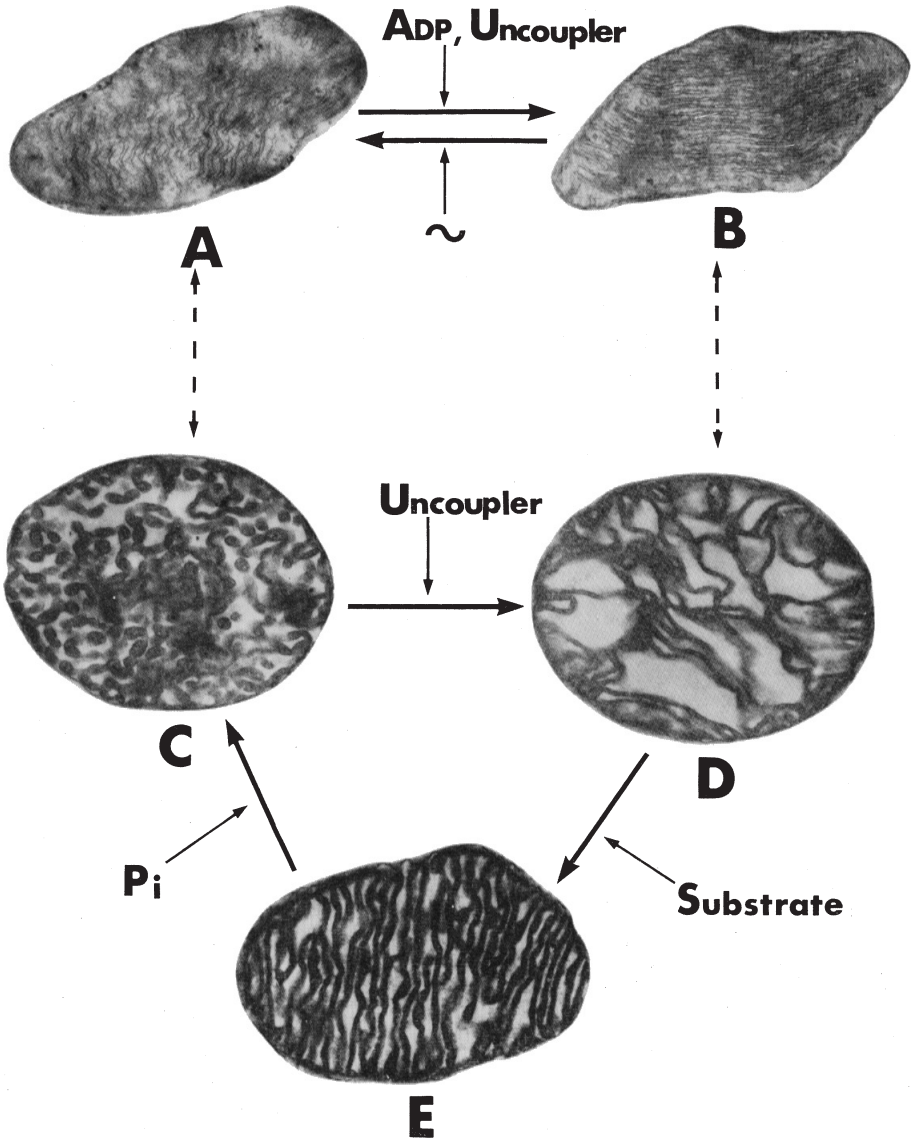


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FIG. 7