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THE EFFECT OF ONCOTIC PRESSURE ON HEART MUSCLE MITOCHONDRIA

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SUMMARY

The role of oncotic pressure (*i.e.* pressure created by non-penetrants of high molecular weight) in structural responses of mitochondria has been studied.

It has been found that treatment of beef of rabbit heart mitochondria by a synthetic non-penetrant of high molecular weight, polyvinyl pyrrolidone, induces a decrease in the intermembrane (intracristal) space and an increase in the matrix space of mitochondria. As a result, the appearance of the *in vitro* mitochondria proves to be similar to that of the *in situ* ones. If a Waring blender is used to homogenize the tissue, only a portion of the mitochondria respond to polyvinyl pyrrolidone. If a glass–Teflon homogenizer is used instead all the mitochondria prove responsive. The addition of 0.5 mM polyvinyl pyrrolidone is found to be sufficient for the effect to be observable.

In the presence of polyvinyl pyrrolidone, energy-dependent changes in mitochondrial structure can be demonstrated. The increase in matrix space by polyvinyl pyrrolidone treatment enlarges even more when an energy source, a penetrating weak acid and a penetrating cation are added. The size of the matrix increases in the following order: (1) de-energized mitochondria without polyvinyl pyrrolidone, (2) de-energized + polyvinyl pyrrolidone, (3) energized + polyvinyl pyrrolidone, (4) as (3) + phosphate ("twisted" configuration of cristae), (5) as (3) + phosphate + Ca^{2+}. Structural changes resembling those indicated in points (2)–(5) are shown for mitochondria in the tissue, when pieces of rat diaphragm muscle treated with an uncoupler, phosphate, and Ca^{2+} were studied in conditions excluding anaerobiosis.

The effect of polyvinyl pyrrolidone is suggested to be due to it balancing the oncotic pressure created by high molecular weight compounds dissolved in the intermembrane water, which are incapable of penetrating the outer mitochondrial membrane. A concept is discussed considering mitochondrial structure changes as a function of the osmotic gradient across the inner membrane and the oncotic gradient across the outer membrane of mitochondria.

Abbreviation: PVP, polyvinyl pyrrolidone.

INTRODUCTION

Even a superficial comparison of the appearances of mitochondria *in situ* and *in vitro* reveals a striking difference between electron micrographs of the two preparations; usually, mitochondria *in vitro* look like a negative of those *in situ* because the intermembrane (intracristal) space is greatly enlarged at the expense of the strongly condensed matrix. The question arises as to what force drives the water into the space between the outer and inner mitochondrial membranes, and squeezes the matrix. It was suggested[1–3] that this force may be the oncotic pressure *i.e.* pressure created by non-penetrants of high molecular weight which are present in the intermembrane space of mitochondria. The outer mitochondrial membrane, being permeable to small molecules, can be a barrier for large molecules, *e.g.* proteins dissolved in the intermembrane water. Therefore, isolation and incubation of mitochondria in sucrose or saline solutions containing no high molecular weight non-penetrants should result in the formation of a colloid concentration difference across the outer membrane, which is favourable for the movement of water into the intermembrane space. If this were the case, addition of a high molecular weight compound to an isotonic sucrose (or saline) solution should balance the oncotic pressure of intermembrane proteins; the effect being followed by a decrease in the intermembrane and an increase in the matrix spaces.

To verify this hypothesis, experiments with a synthetic, water-soluble polymer, polyvinyl pyrrolidone (PVP) have been performed. It was found[1–3] that addition of PVP to rabbit heart muscle mitochondria induces a decrease in intermembrane space and an enlargement of the matrix. Independently, a similar effect was observed recently by Harris *et al.*[4] who studied rat liver mitochondria and applied some natural polymers (serum albumin, ficoll and dextran).

This paper summarizes the results of further investigations of the effects of PVP on beef and rabbit heart muscle mitochondria. The experiments showed that 0.5 mM PVP is required to reproduce the effect, thus indicating that the protein concentration in the intermembrane space is close to this level. Energy-dependent changes in the morphology of mitochondria, incubated in the medium with PVP have been demonstrated. Similar structural changes were observed in diaphragm muscle mitochondria *in situ*.

EXPERIMENTAL

Beef heart mitochondria were prepared by the slightly modified method of Crane *et al.*[5] (see ref. 6). For the preparation of rabbit heart mitochondria, a similar procedure was employed but instead of a Waring blender a glass–Teflon homogenizer was used.

For studying diaphragm muscle, a piece of the tissue (about 40 mm^2) was fastened in a Teflon frame and put into a polarographic cell or a spectrophotometer cuvette . The respiration and cytochrome spectra of the diaphragm piece were measured as described previously[7].

Electron microscope studies were carried out using a Hitachi HU-11B microscope. Samples were fixed with 5% glutaraldehyde and treated with OsO_4, alcohols,

uranyl acetate and epoxide resin 812. Thin sections of mitochondria and tissues were prepared with an LKB-4800 ultramicrotome.

RESULTS

The morphology of heart muscle mitochondria *in situ* and *in vitro* are compared in Fig. 1. Electron micrograph A demonstrates mitochondria in beef heart tissue

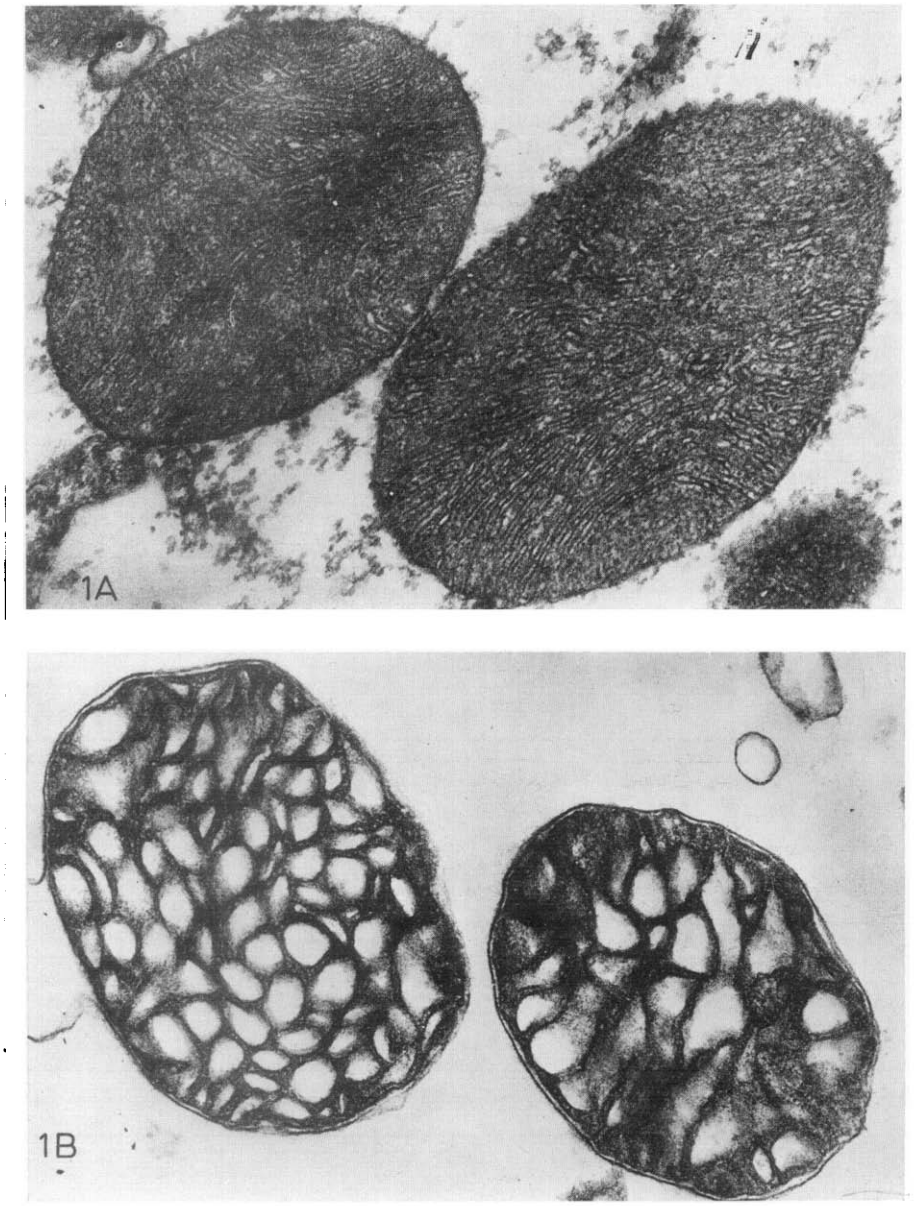


Fig. 1. Comparative morphology of beef-heart mitochondria *in situ* (A) and *in vitro* in 0.25 M sucrose solution (B). Magnification × 71 200.

(a part of the tissue without myofibrill, where mitochondria are freely "suspended" in cytosol, was chosen). Isolated beef heart mitochondria (heavy fraction) suspended in isotonic (0.25 M) sucrose solution are shown in Micrograph B. One can see that the intermembrane (intracristal) space of the *in vitro* mitochondria is strongly enlarged, and the matrix space reduced, in comparison with the *in situ* state.

In Fig. 2 the effect PVP on the appearance of mitochondria from beef heart is shown. It is seen that the mitochondrial population washed and suspended with 0.25 M sucrose + 5 % PVP (Micrograph B) contains a fraction of the mitochondria whose appearance proves to be similar to that of mitochondria *in situ*. These mitochondria are characterized by the highest electron density, an increased matrix and a decreased intermembrane (intracristal) space. The same population also includes two other types of mitochondria: one resembling "heavy" mitochondria without PVP and the other resembling "light" mitochondria. The latter show the lowest electron density of the matrix and loss of the inner membrane integrity. The mitochondrial population without PVP (Photograph A) contains no mitochondria of the *in situ* appearance.

Heterogeneity of the mitochondrial population *in vitro* seems to be a result

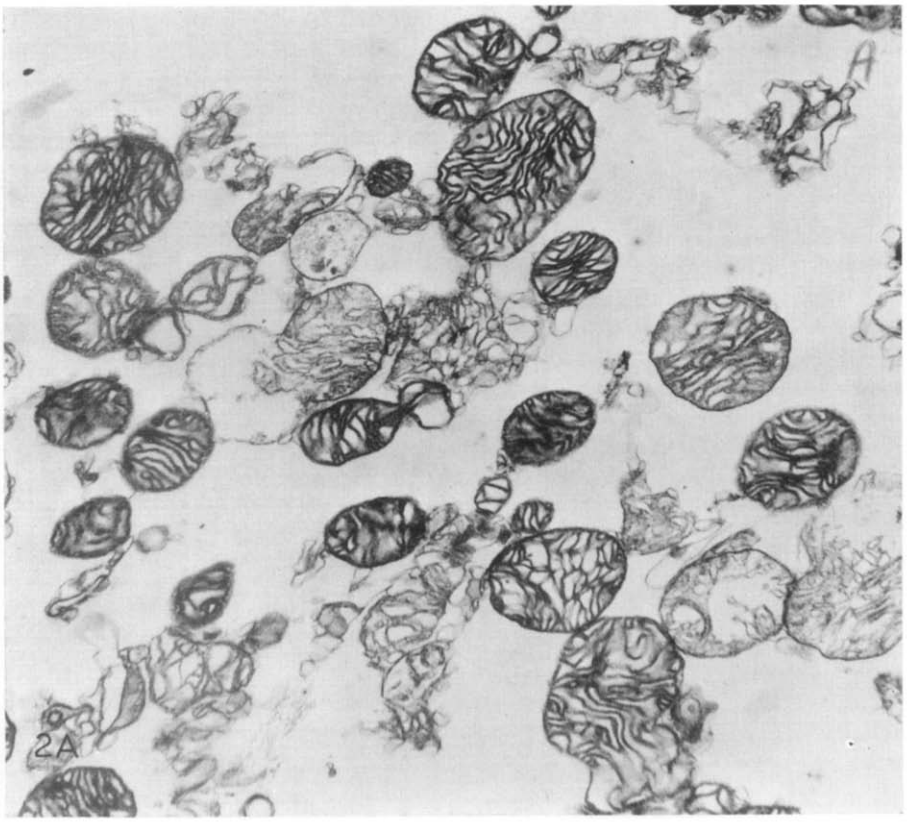


Fig. 2A. The effect of polyvinyl pyrrolidone on the appearance of beef-heart mitochondria. Mitochondria *in vitro* washed twice and suspended in 0.25 M sucrose.

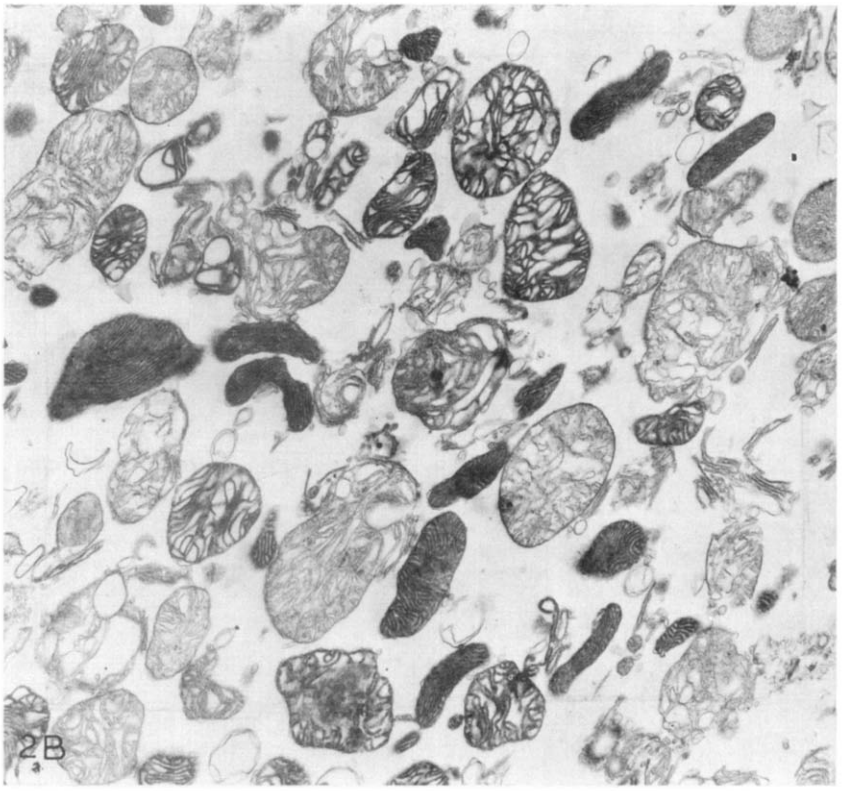
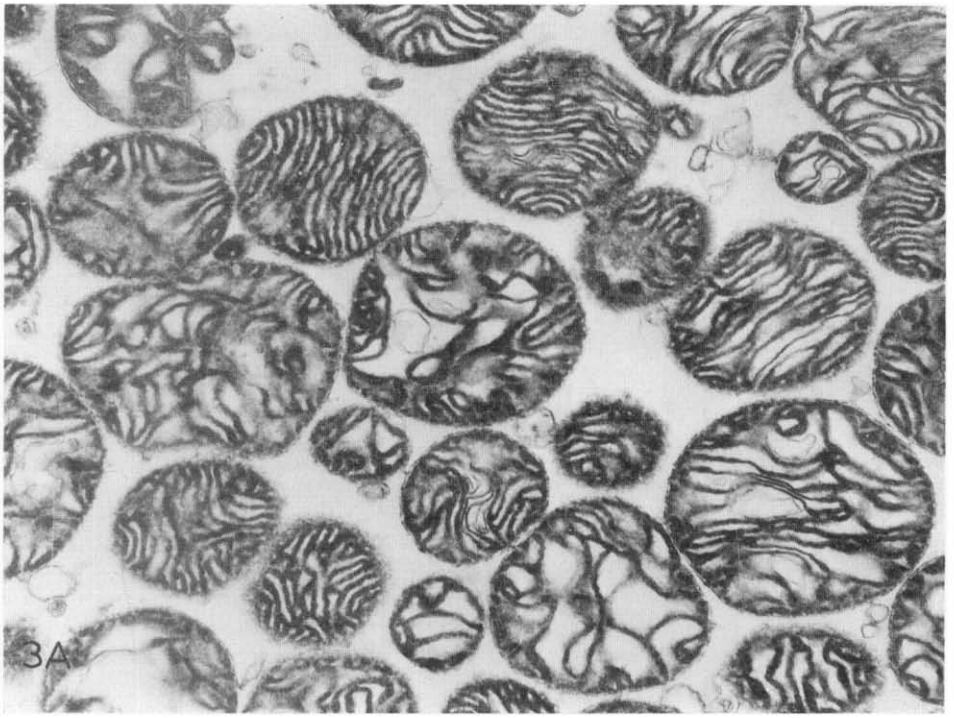


Fig. 2B. As (A) but the solutions for washing, suspending and fixing were supplemented with 5% PVP. Homogenization was performed with a Waring blender. Magnification × 16800.

of damage to the mitochondrial membranes during homogenization in the Waring blender. If a glass–Teflon homogenizer, and not a blender, was used, almost all the mitochondria prove to be sensitive to PVP. This is demonstrated in Fig. 3 showing the concentration dependence of the PVP effect. It is seen that the mitochondrial population responds to PVP in a uniform fashion. Characteristic changes in the mitochondrial morphology are detectable at a PVP concentration of 0.5 %. Isolated mitochondria regain their *in situ* appearance when treated with 2 % (0.5 mM) PVP.

In further experiments it was found that the energy-dependent changes in the ultrastructure of mitochondria can be demonstrated in the presence of PVP. One can see (Fig. 4) that the intermembrane (intracristal) space decreased and the matrix space increased in the following order: mitochondria de-energized by the uncoupler, energized, energized + phosphate and energized + phosphate + Ca^{2+}. The configuration of the cristae in the energized state + phosphate resembles that of the twisted state of mitochondria incubated in solutions without polymers[8–10].

Similar structural responses were demonstrated *in situ*. Consequently, pieces of rat diaphragm muscle were used. A piece of muscle was fastened in the Teflon frame and incubated in a polarographic cell for 5 min in the reaction mixture of the composition indicated in the legend to Fig. 5. After 5 min of incubation the samples were fixed with a mixture of the same medium supplemented with glutaraldehyde.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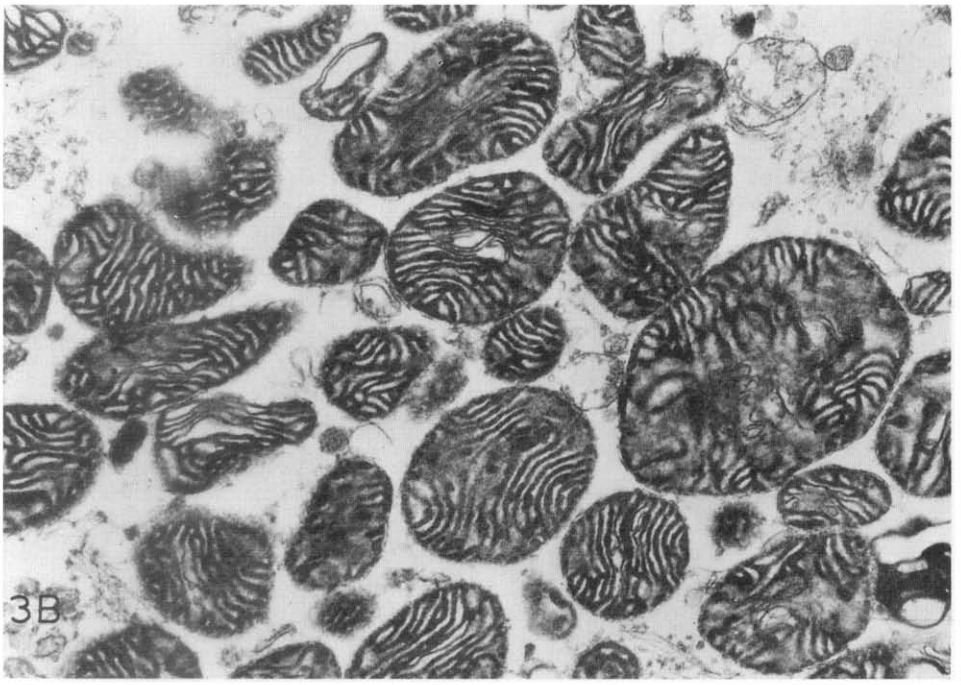
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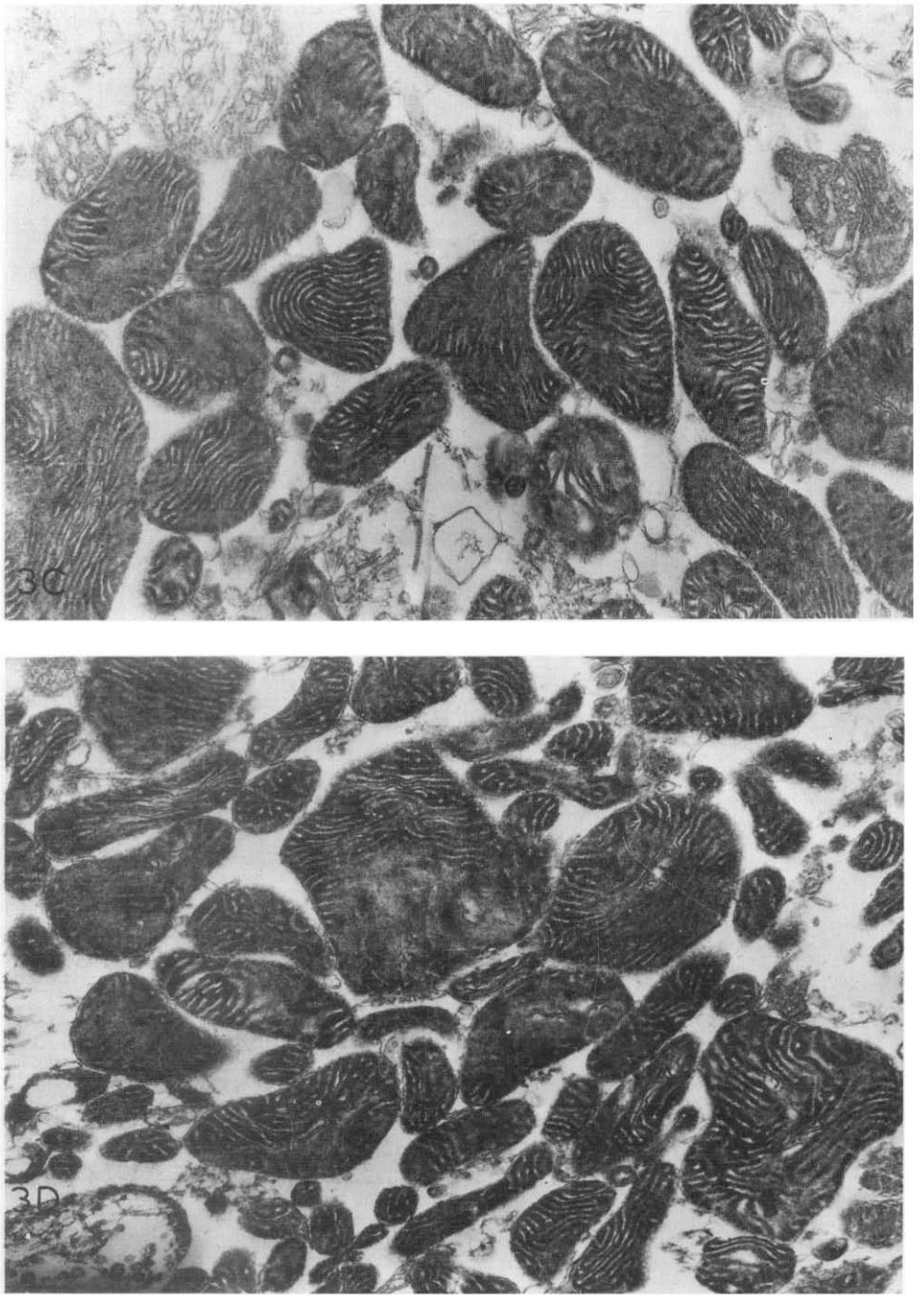
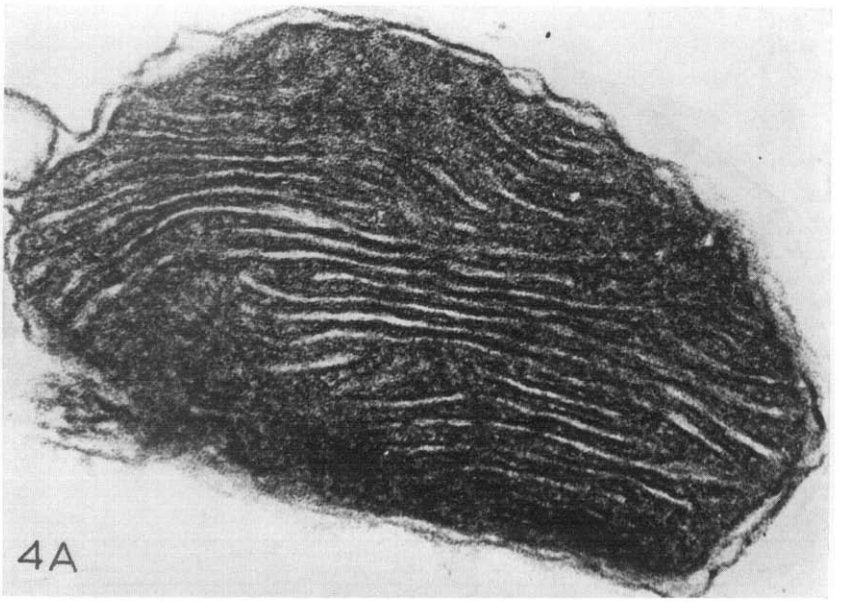
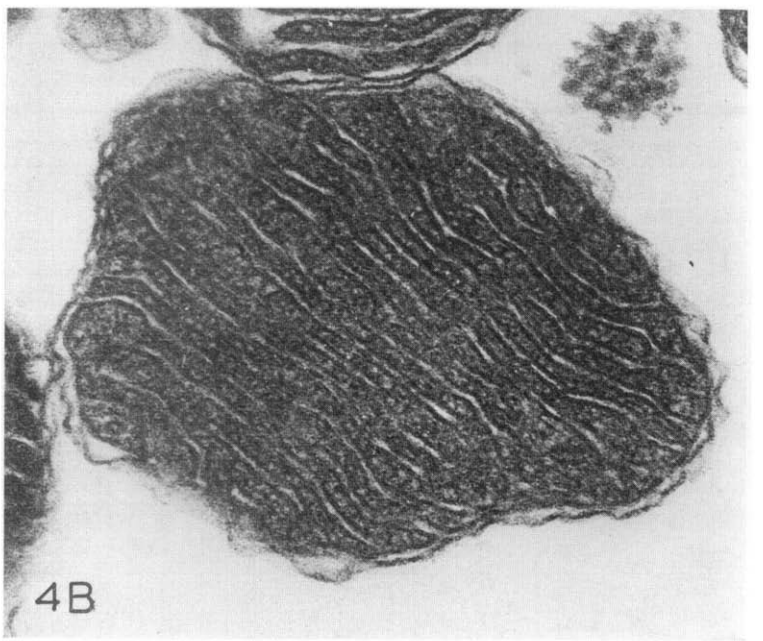
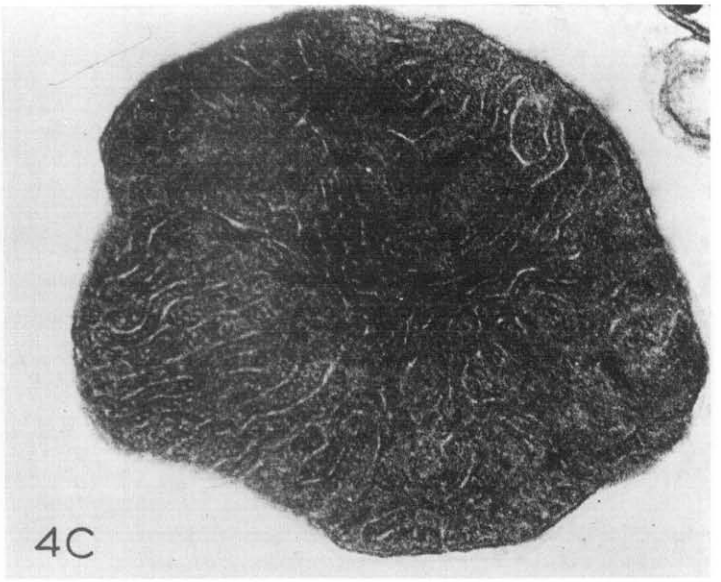


Fig. 3. PVP concentration-dependent changes in the ultrastructure of rabbit-heart mitochondria. Rabbit-heart mitochondria were isolated in 0.3 M sucrose + 10 mM Tris–HCl, pH 7.5, washed twice and suspended in the same solution without (A) or with (B–D) PVP. Concentration of PVP: B, 1%; C, 2%; D, 5%. Glass–Teflon homogenizer. Magnification × 35100.

As is demonstrated by the micrographs of Fig. 5, the addition of an uncoupler, phosphate and Ca^{2+} to the tissue induces changes in the appearance of mitochondria in the same way as those *in vitro* in the presence of PVP. The lower electron density and larger volume of the matrix space of mitochondria *in situ* in samples without added phosphate (Fig. 5B) and Ca^{2+} (Fig. 5C) in comparison to those *in vitro*







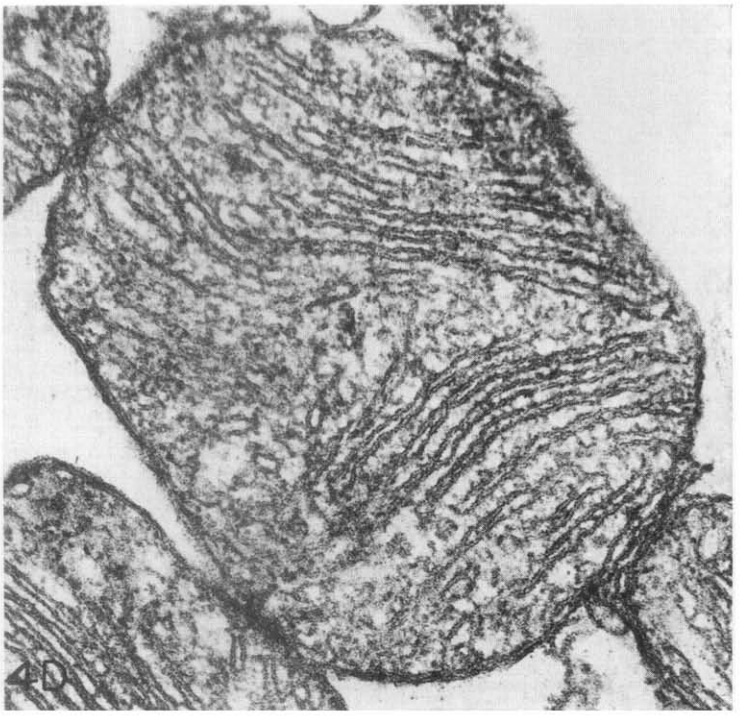
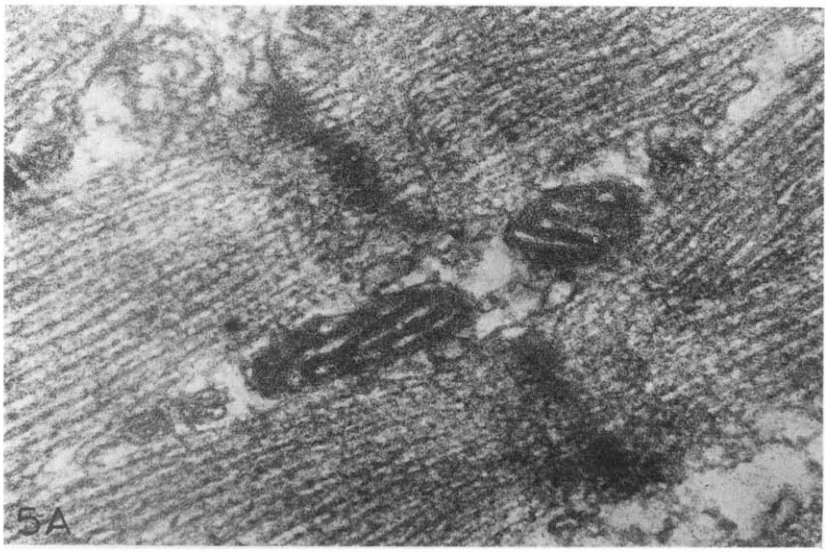
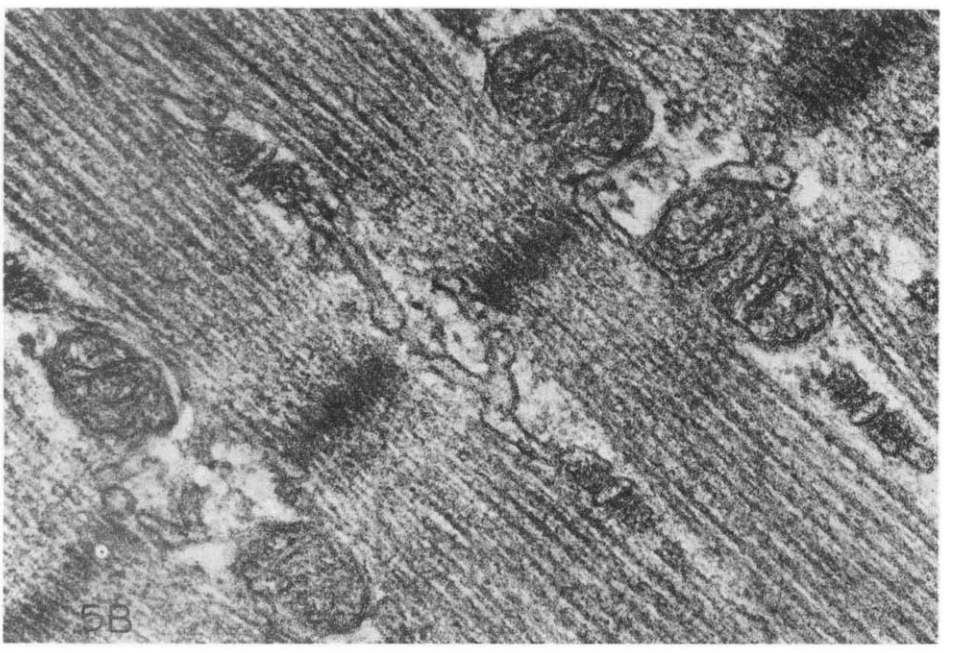


Fig. 4. Energy-dependent changes in mitochondrial ultrastructure in the presence of PVP. Heavy beef-heart mitochondria were prepared as in Fig. 2B and incubated in 0.25 M sucrose + 10 mM Tris–HCl, pH 7.5 + 5% PVP for 10 min at room temperature with vigorous stirring. Additions to incubation mixture: (A) 10 mM succinate, 5 mM potassium phosphate, pH 7.5, $5 \cdot 10^{-6}$ M *p*-trifluorometoxycarbonylcyanide phenylhydrazone; (B) 10 mM succinate; (C) 10 mM succinate and 5 mM potassium phosphate; (D) as (C) + 1 mM $CaCl_2$. Magnification × 93000.

(Figs 4B, 4C) may be due to the presence of endogeneous phosphate and Ca^{2+} in muscle tissue.

It should be noted that the *in situ* responses could be revealed only under aerobic conditions. Anaerobiosis always resulted in de-energization, so that the effects of uncouplers and ions on the mitochondrial morphology were impossible to demonstrate. To exclude the development of oxygen deficiency, the reaction mixture was saturated with oxygen before the experiment, and the concentration of oxygen in





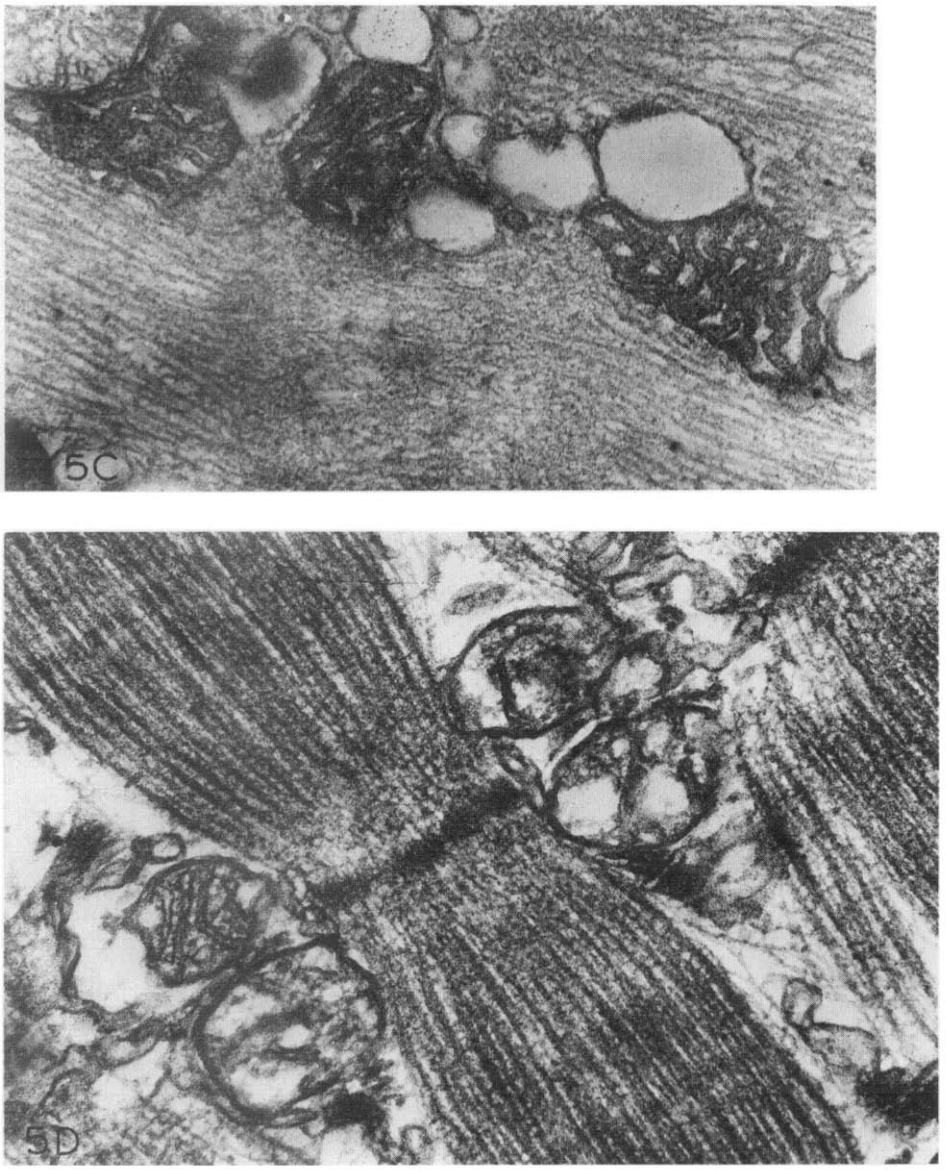


Fig. 5. Energy-dependent changes in ultrastructure of mitochondria in rat diaphragm muscle *in situ*. Pieces of rat diaphragm fastened in the Teflon frame were incubated at room temperature with solutions of the following composition: (A) 0.145 M NaCl, 5.6 mM KCl, 20 mM Tris–HCl, pH 7.5, $1.6 \cdot 10^{-4}$ M 2,4-dinitrophenol; (B) as (A) but without 2,4-dinitrophenol; (C) as (B) + 10 mM potassium phosphate, pH 7.5; (D) as in (B) + 5 mM $CaCl_2$. Gas phase, O_2; after 5 min incubation the diaphragm pieces were fixed by the addition of 5% of glutaraldehyde. Magnification × 77 400.

the reaction mixture was followed polarographically during the incubation. It was found that in the conditions used the piece of diaphragm in the absence of 2,4-dinitrophenol consumed only a small portion of the oxygen dissolved in the incubation mixture. Addition of 2,4-dinitrophenol induced a two-fold stimulation of

the respiration rate, but even in this case the main portion of the oxygen had not been utilized by the end of the incubation. A special spectrophotometric control showed that cytochrome oxidase and myoglobin in the diaphragm remained oxidized throughout the period of incubation.

DISCUSSION

The interplay of oncotic and osmotic pressures affecting the mitochondrial morphology is schematically shown in Fig. 6. It is suggested that a high concentration of large non-penetrants in cytosol is responsible for water extrusion from the intermembrane (intercristal) space in the *in situ* mitochondria. As a result, the matrix space proves to be large, and the intermembrane space to be small (Scheme A in Fig. 6). The same situation takes place *in vitro* when mitochondria are incubated with a high molecular weight compound.

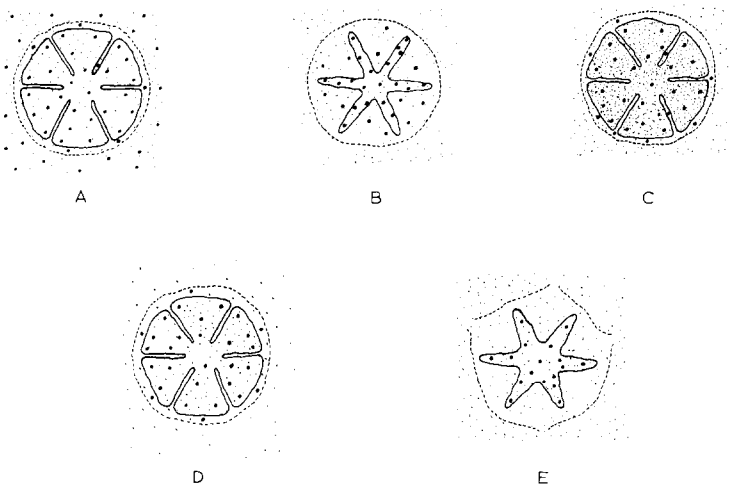


Fig. 6. A scheme illustrating the effect of the oncotic pressure in the intermembrane space and osmotic pressure in the matrix on the mitochondrial structure. Small points – compounds of low molecular weight which penetrate the outer but not the inner mitochondrial membrane; large points – compounds of high molecular weight which cannot penetrate either the inner or the outer mitochondrial membranes. For explanations of States A – E, see the text.

Incubation of mitochondria in isotonic solution without large non-penetrants causes a water influx into the intermembrane space, the effect resulting in a dilution of the high molecular weight compounds confined in the intermembrane space. In this case, the intermembrane space expands and the matrix diminishes (Scheme B).

A reduction of the intermembrane space can be induced by water influx into the matrix. This can be achieved by (1) an increasing concentration of low molecular weight compounds in the matrix (*e.g.* the energy-dependent accumulation of a penetrating cation and a weak acid) (Scheme C), or (2) by a decreasing concentration of low molecular weight compounds in the extramitochondrial space (Scheme D).

If the outer membrane is burst open (Scheme E), oncotic pressure stops affecting the mitochondrial structures, and the size of the matrix is now determined

only by osmolarities on both sides of the inner membrane and the rigidity of this membrane.

The results summarized above as well as some data from other laboratories are in agreement with the concept presented in Fig. 6.

(1) Addition of large non-penetrants, both natural[4] and unnatural (see refs 1–3 and this paper, Figs 1–4), to the isotonic incubation mixture increases the matrix and decreases the intermembrane space of the mitochondria (transition from State B to State A, see Fig. 6), the effect similar to those of energy-dependent cation + weak acid loading (C→A transition) of or incubation in a hypotonic mixture (D→A transition).

(2) Submitochondrial particles as well as mitochondria deprived of their outer membrane ("mitoplasts") do not respond to a large non-penetrant treatment by the above changes in their structural organization[11].

(3) The population of mitochondria prepared after the Waring blender homogenization includes a fraction of mitochondria demonstrating no PVP response, although the inner membrane seems to be intact as judged from the high density of the matrix. Apparently, this fraction represents mitochondria with a damaged outer membrane (Fig. 6, Scheme E).

Accepting the idea of oncotic pressure as a factor affecting mitochondria, one ought to consider two questions.

(1) What is the role of the possible changes resulting from oncotic pressure in the intermembrane or in permeability of the outer mitochondrial membrane in different structural and functional responses of mitochondria?

(2) To what extent can the data obtained with isolated mitochondria, which were always studied in mixtures without large non-penetrants, be extrapolated to the *in vivo* conditions where the cytosol concentration of large non-penetrants is rather high?

The data of this paper indicate that four main structural states of mitochondria (de-energized, energized, energized-twisted and swollen) can still be demonstrated in the presence of a large non-penetrant, PVP (Fig. 4). This fact indicates that the energy-dependent structural responses mentioned do not result from changes in the outer membrane or intermembrane space of mitochondria. Similar states were shown in the *in situ* mitochondria, as is seen in experiments with rat diaphragm muscle in controlled aerobic conditions (Fig. 6). Some of these *in situ* responses were demonstrated by other authors (see refs. 12–14). It should be stressed that energy-dependent changes in mitochondrial morphology *in situ* may be observed only if respiration is not limited by the rate of oxygen diffusion from the incubation mixture into the tissue. It seems very probable that some discrepancies in the results of various laboratories dealing with the *in situ* energy-dependent changes of mitochondria are due to an oxygen deficiency of a different degree (see refs 12–14). Some effects of PVP, included in the isolation mixture, on the biochemical properties of mitochondria were mentioned by Novikoff[16], who was the first to observe the the favourable action of PVP on mitochondrial morphology. Similar action of the colloid was mentioned by Birbeck and Reid[17] who used dextran instead of PVP.

Important observations on the effect of oncotic pressure on mitochondrial structure and functions was published quite recently by Harris *et al.*[4]. The authors showed that large natural non-penetrants not only induce a change in the appearance

of rat liver mitochondria but also induce a large decrease in the permeability of the inner mitochondrial membrane to pyruvate and all the Krebs cycle substrates excluding malate. As a result, malate proved the only extramitochondrial substrate capable of supporting the high rate of respiration of mitochondria in the reaction mixture supplemented with a compound of large molecular weight. The latter findings demonstrate that some essential metabolic characteristics of mitochondria incubated *in vitro* without colloids can strongly differ from those in the presence of colloids. Since (1) the morphology of mitochondria incubated with large non-penetrants is similar to that of the *in situ* mitochondria (see refs 1–4, 15 and this paper) and (2) cytosol contains soluble proteins and other colloids, one may think that native properties should be inherent to the mitochondria incubated *in vitro* with a colloid rather than without it.

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