Protamine Induced Configurational Changes in Paracrystalline Structures from Heat Desintegrated Mitochondria

It has been suggested that the 'crystal-like structure' formed by heat-induced rearrangements of mitochondrial membranes could play a role in ultrastructural transitions in intact mitochondria[1-3]. This supposition was examined in the present study using protamine – known potent inducer of structural changes in intact mitochondria[4-6]. It was observed that paracrystalline structures undergo rapid ultrastructural transitions upon addition of protamine, and the direction of changes is the same as that observed in intact mitochondria.

Materials and methods. Rat liver mitochondria were prepared according to WEINBACH[7] in 0.25 *M* sucrose with 3 m*M tris* chloride pH 7.3. Protein content was estimated by the biuret method[8]. 0.1 ml of mitochondrial suspension containing 6 mg of mitochondrial protein was added into the 1 ml of pH 7.3 medium containing 15 m*M* KCl, 5 m*M* $MgSO_4$, 50 m*M tris* chloride, 5 m*M* potassium phosphate and 7 m*M tris* succinate. In the experiment No. 2, the medium contained also 500 µg of protamine. After 45 sec of incubation of samples at 25 °C, the tubes were transfered into the boiling water bath, heated 5 min, cooled 15 min at room temperature, and then centrifuged at 10,000 × *g* for 40 sec. Before centrifugation, the tubes with experiment No. 3 and No. 4 were supplemented by addition of 500 µg of solution of protamine. After centrifugation, pellet obtained was fixed with solution of 2% osmium tetroxide buffered with 0.21 *M* potassium phosphate pH 7.4 containing 50 m*M* sucrose, at 0 °C for 1 h. The pellets were stained with 1% uranyl acetate in 25% ethanol, dehydrated with ethanol and acetone and embedded in Epon 812. The specimens were examined in a JEM 7A electron microscope at 80 kV.

Results and discussion. Various explanations have been offered to account for a mechanism involved in configurational changes in mitochondria. In accordance with elegant experiments of KATCHALSKY and ZWICK with 'polyelectrolytes'[9], and in accorance with chemiosmotic hypothesis – it is postulated that Δ pH across the mitochondrial membrane play an important role in ultrastructural transitions in mitochondria[10]. On the other hand, there are experimental observations that similar structure as observed in energized conditions can be induced by addition of non-penetrant polyanions as phosphotungstate[11], and the same may be achieved by penetrant anions in the presence of transport inhibitor butylmalonate[6]. These facts suggest that ultrastructural configuration of mitochondria described as 'energized' could be related to the 'asymetry' in the anion distribution on both sides of mitochondrial membrane. The other possibilities that the configurational changes could be organized either at the level of mitochondrial matrix as proposed by HACKENBROCK[12], or at the level of mitochondrial membrane as has been porposed from GREEN's laboratory[13, 14], seem to be at the present time difficult to examine by experiment.

In the present paper we should like to show some experiments, which in our contention support the idea that the ultrastructural transitions in mitochondria could be organized at the level of mitochondrial membrane by the direct interaction of inducers with structural elements of mitochondrial membrane, which, according to KORMAN et al.[15], are organized in paracrystalline lattice structures.

Recently we observed[1-3] that heating of mitochondrial suspension of beef heart, or rat liver mitochondria leads to the disintegration of mitochondrial structure with formation of new paracrystalline structures. These 'crystal-like' structures of heated rat liver mitochondria are also presented in Figure 1.

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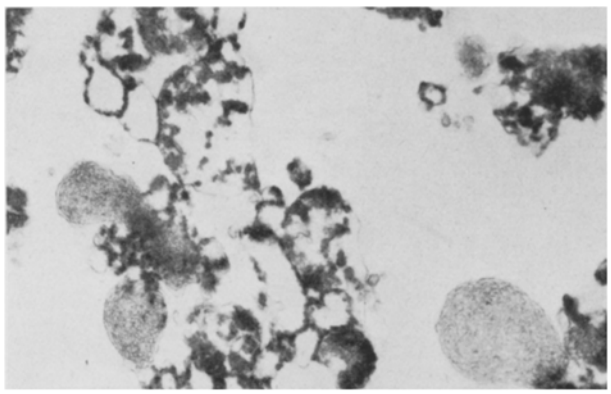

Fig. 1. 'Crystal-like' structures in rat liver mitochondria observed after boiling (5 min) of mitochondrial suspension (exp. 1). × 17,000.

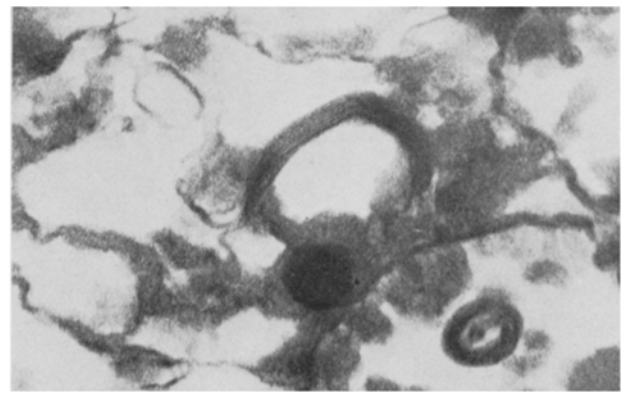

Fig. 2. Inhibitory effect of protamine on formation of 'crystal-like' structures. Protamine was added before boiling (exp. 2). × 86,000.

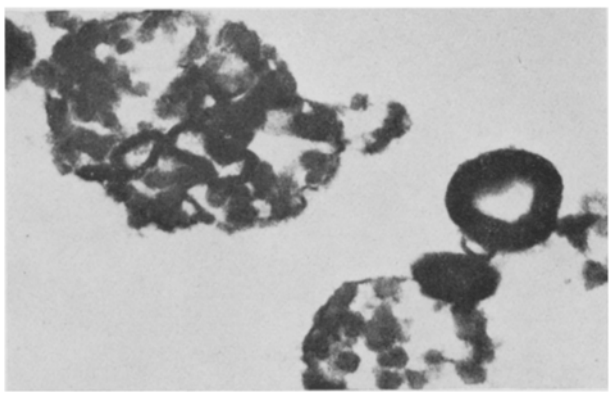

Fig. 3. Protamine induced configurational changes in 'crystal-like' structures. Protamine was added into the boiled mitochondria (exp. 3). ×34,000.

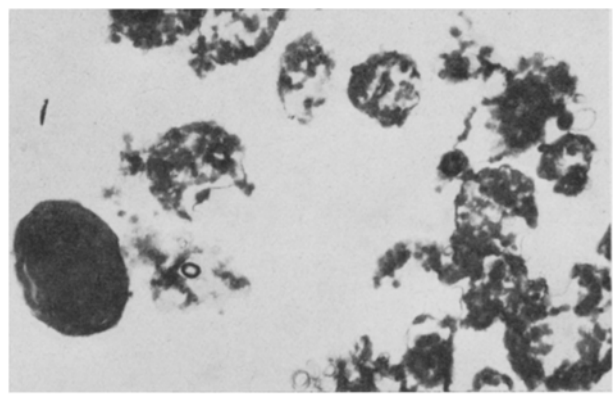

Fig. 4. Experiment 4 as in experiment 3. ×15,500.

The possibility that this 'membrane derived' paracrystalline material can play a role in ultrastructural transtions in mitochondria was examined electron microscopically by observations of interaction between 'crystal-like' structures and protamine.

As is shown in Figure 2, protamine prevented formation of 'crystal-like' structures when added to the mitochondrial suspension before boiling. Protamine added into the heated mitochondria induced ultrastructural transitions in paracrystalline structures already formed (Figure 3 and 4). In all experiments with protamine, occurence of typical 'myelin figures' can be detected, instead of typical for heated mitochondria 'crystal-like' structures. It is interesting that similar changes occured when protamine was repleaced by cytochrome c (not shown).

The direction of ultrastructural transitions observed is the same as that reported in intact mitochondria upon addition of protamine, visualized as a strong contraction of all membranous material in mitochondria[4–6]. It is possible that the substance interacting with protamine in intact mitochondria is the same as is visualized as a paracrystalline material after boiling. Observations that upon addition of protamine this 'membrane derived' paracrystalline material could form 'myelin structures', suggest that protamine is incorporated into paracrystalline material. This binding is probably related to the presence of lipids. This suggestion is in accordance with observations of RAND and SENGUPTA[16] of formation of lattice structures by cardiolipin, and is confirmed by our observations that similar paracrystalline structures as observed in boiled mitochondria were visualized in intact, unboiled beef heat mitochondria incubated with cardiolipin[2,3].

Binding of protamine into structural elements of membrane of intact mitochondria would change the value of proton motive force (PMF) as well as a ratio of $\Delta\Psi/\Delta$pH in PMF, leading to the inhibition of respiration. This inhibition we found to be reversed by energy 'releasing systems': ATP synthesis or action of uncouplers[17,18].

Résumé. La protamine provient de structures paracrystallines, résultant de la décomposition thermique des membranes mitochondriennes. Après la formation de ces structures, l'addition de la protamine les rend miéliniques.

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On the Ultrastructure of Mammalian Tendon

The collagen fibers which are the major structural component of rat tail tendon (RTT) have been noted for some time to follow some wavy course through the tendon bundle, and become straight and parallel to the tendon axis only when the tendon is stretched[1]. Different workers, using different techniques, have characterized the waveform variously as planar or helical, either with or without intertwining of the fibers. Recently, DIAMANT, KELLER et al.[2] analyzed the shape of the waveform in RTT in considerable detail by the straightforward technique of polarizing microscopy, and demonstrated that it is a planar wave shape. When the tendon was teased down to fine bundles, it was observed that the physical outlines of these sub-bundles followed the waveform as that deduced from the polarizing optics of the intact tendon bundle. They also observed that straightening of the waveform, rather than extension of the collagen fibers themselves, is the principal mechanism of deformations in the range expected in vivo, and deduced that the independent me-

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