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The Formation of Intracristal Structures Induced in Skeletal Muscle Mitochondria by High Pressure

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Abstract

Following the application of high pressure to skeletal muscle for extended periods, intracristal structures are found in the mitochondria. In addition, electron dense granules of 70-170 nm diameter are found in the matrix of these mitochondria. In contrast, pressure-treated liver mitochondria show only large (300-400 nm) matrix granules but not the intracristal structures. Both the inner and outer mitochondrial membranes appear intact after pressure-treatment. Short periods of pressure-treatment have little effect on either the morphology of mitochondria or the pH of the tissues. It is suggested that the formation of the intracristal structures may be due to the effects of pH rather than pressure alone. This finding raises the possibility that intracristal structures may occur as a preparative artefact particularly where the tissue has undergone considerable manipulation.

Introduction

A recent review [1] of the effect of pressure on biological systems shows that while considerable attention has been directed towards factors involved in the maintenance of cell shape, references to pressure effects on mitochondria are sparse. This may indicate that few effects have been noted in the course of examination of other structures.

In the present paper, we wish to report the formation of intracristal structures within the mitochondria of skeletal muscle which has been

subjected to pressure treatment. Liver mitochondria are compared to those of skeletal muscle in their response to this treatment.

Recently Hall and Crane [2, 3] have drawn attention to the presence of a structural element in the intracristal space of isolated beef heart mitochondria, the presence of which was independent of the conformation of the mitochondria. It was shown by serial sectioning that the intracristal material consisted of sheets of parallel rods of 5-7.5 nm diameter and it was suggested that it might serve in some structural capacity. The intracristal material has been further investigated [4] and it was shown that a lattice structure could be induced by treatment of the isolated mitochondria with phosphotungstic acid.

Methods

Samples of three different muscles (semimembranosus, semitendinosus and adductor) and liver were obtained from sheep within 30 min of slaughter. Portions of each tissue, sufficient to fill a 7-ml glass culture bottle, were taken and the bottles sealed with a serum cap. These were pressurized, using water as the hydraulic fluid, in a stainless steel pressure vessel immersed in a constant temperature bath at 10° C. The pressure was held at 15,000 psi for 5 and 60 min. After the pressure was released small pieces of tissue were fixed [5] for 2 h at 0° C in a mixture of 2.5% glutaraldehyde and 1% osmic acid in 0.1 M cacodylate buffer pH 7.2. Control tissues were held for 5 and 60 min at 10° C and then fixed in exactly the same way.

After fixation, the tissues were washed overnight in 0.1 M cacodylate buffer, dehydrated in a graded alcohol series and embedded in Araldite. Sections cut with glass knives were stained with a saturated uranyl acetate solution in 50% alcohol and then stained with lead citrate [6]. The sections were examined in a Philips EM 300 electron microscope.

To determine the pH of the tissues, approximately 1 g of tissue was homogenized in 15 ml of 5 mM iodoacetic acid and the pH of the homogenate measured using a glass electrode.

Results

Figure 1 shows the appearance of muscle subjected to a pressure of 15,000 psi for 60 min. The mitochondria show the presence of electron-dense material in the intracristal space as well as dense granules in the

Figure 1. Semitendinosus muscle held at 15,000 psi for 60 min at 10° C. Mitochondria show the presence of both intracristal structures and matrix granules. Myofilaments (mf) surround the mitochondria (×62,000).

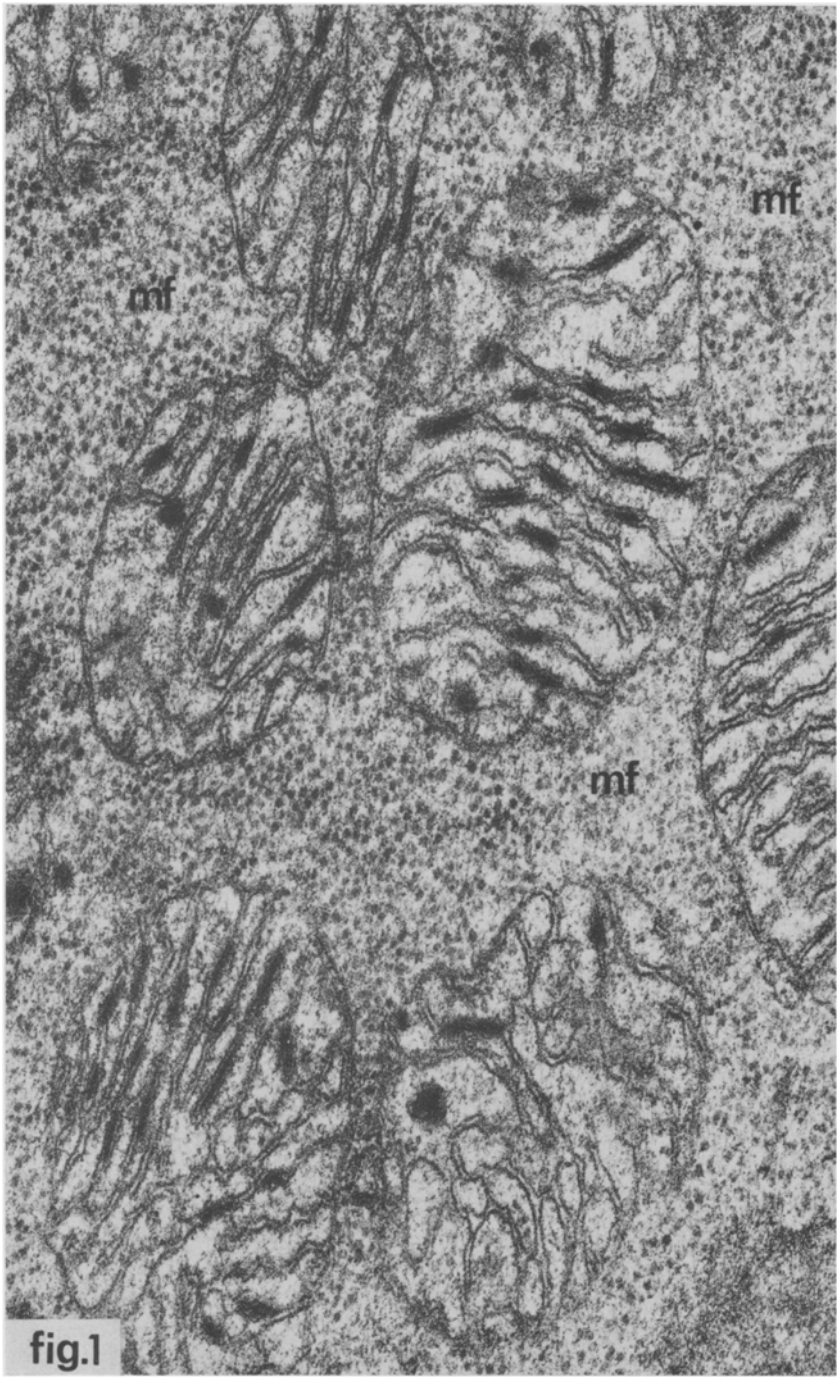


fig.1

mitochondrial matrix. The mitochondria in these pressure-treated muscles are partly swollen and have lost some matrix density but for the most part both the internal and external membranes appear intact. As seen in Fig. 1, most of the mitochondria show the presence of intracristal material but not all the cristae within an individual mitochondrion are involved.

The most common form of intracristal structure is a bar of about 12 nm diameter running between the cristal membranes but not occupying the full width of the crista (Fig. 2). No difference was noted in the response to treatment of the three different muscles used.

Up to three dense granules could be found in a section of a mitochondrion and these granules varied in size from 70 to 170 nm diameter. Examination of serial sections (not shown) revealed that the matrix granules were not derived from the rupture of the cristae followed by release of intracristal material into the matrix. Examination of the control muscles did not reveal the presence of either intracristal material or dense granules in the matrix of the mitochondria.

In some instances the intracristal material shows a high degree of organization as in Fig. 3. The centre to centre distance between the round structures is 15 nm while a near longitudinal section (inset Fig. 3) shows that these structures are rod-like in appearance. It has been demonstrated by Hall and Crane [3] that the precise dimensions of the intracristal structures are determined by the method of fixation. Furthermore it has been shown [4] that the configuration of the intracristal material can be influenced by their treatment. The shorter periods of pressure treatment of muscle (5 min) did not lead to the formation of either the intracristal structures or the matrix granules (Fig. 4), nor were these structures seen in the control tissues (Fig. 5).

Under the same experimental conditions as muscle, no intracristal structures were found in pressure-treated liver, but instead a single large matrix granule of 300-400 nm diameter was present in most mitochondria (Fig. 6). Shorter pressure treatment of liver had no marked effect on the mitochondria as compared to those of the control tissue, except that occasional matrix granules were seen (Fig. 7). While the 60-min pressure treatment clears most of the mitochondrial matrix, there is little indication of gross damage to either the inner or outer mitochondrial membranes. It may be noted in passing that sheep liver

Figure 2. Detail of intracristal structure in mitochondria of semimembranosus muscle held at 15,000 psi for 60 min. Note the clear matrix of the mitochondrion (x52,000).

Figure 3. Semimembranosus muscle after pressure treatment for 60 min showing mitochondrion with intracristal material organized into rods seen transversely section (x62,500). Inset shows the rods obliquely sectioned in the direction of the arrows (x69,000).

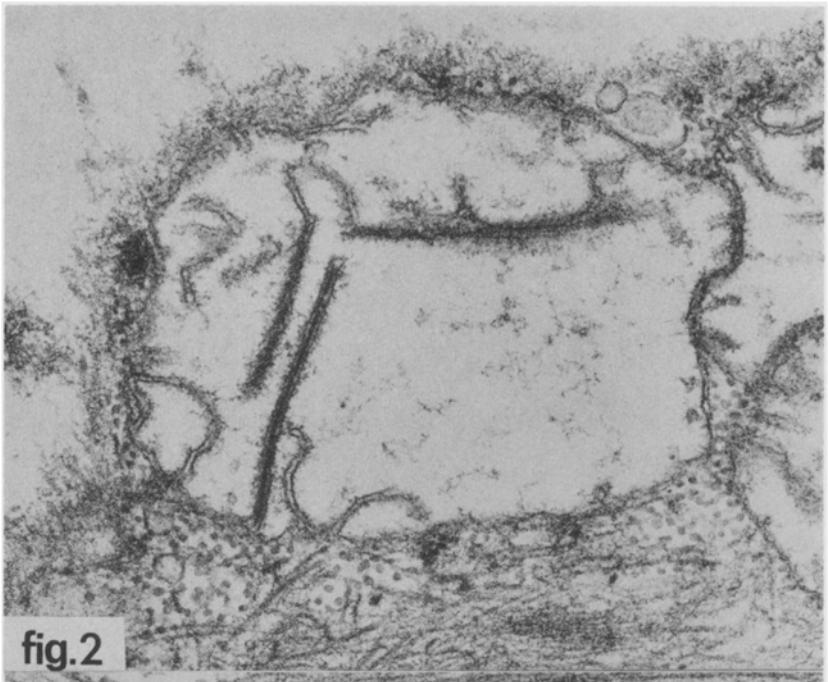


fig.2

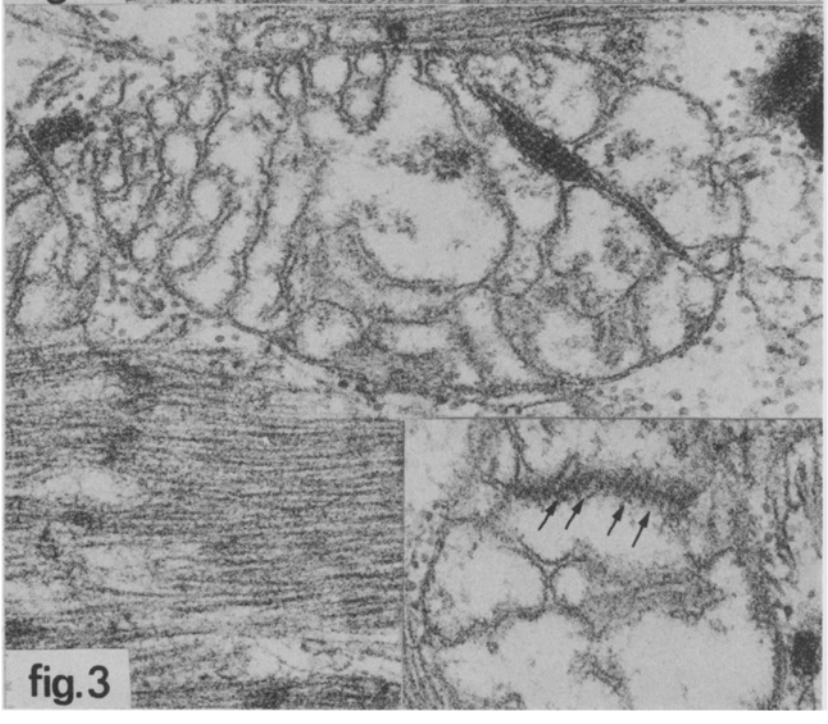


fig.3

mitochondria do not have an assembly of cristae as extensive as those in mitochondria from other species.

Table I shows the pH of these tissues after pressure treatment and while pressure has little effect upon liver as compared with the control, there is a substantially lower muscle pH as a result of the 60-min pressure treatment.

TABLE I. Effect of 15,000 psi pressure on tissue pH

Sample	Time (min)	pH Control	pH Pressurized
Liver	5	6.31	6.31
	60	6.22	6.22
Muscle	5	6.59	6.34
	60	6.49	5.65

Discussion

These experiments show that it is possible to induce the formation of intracristal structures in skeletal muscle mitochondria by the application of high pressure to the tissue. However, since these structures are not found after a short exposure to pressure, it is likely that factors other than pressure are also involved in their formation. One possibility is that the formation of these structures is reversible upon the release of pressure unless some associated change such as a lower pH "fixes" the tissue in an altered configuration. The only available evidence is from the work of Landau and Thibodeau [7] who fixed *Amoeba proteus* under pressure and also after the pressure had been released. The fact that no differences can be seen in the mitochondrial morphology of mitochondria fixed by either of these methods may indicate that such reversible pressure-induced morphological changes in mitochondria do not occur.

A more likely explanation is that it is the low pH which is responsible for the formation of these intracristal structures and the role of the pressure treatment is to accelerate glycolysis and hence lower the pH of

Figure 4. Adductor muscle after pressure treatment for 5 min shows no formation of intracristal structures of matrix granules but a loss of matrix density as compared to the control (×62,000).

Figure 5. Control adductor without pressure treatment (×51,500).

Figure 6. Liver mitochondrion showing large matrix granule and low matrix density. Held at 15,000 psi for 60 min (×54,000).

Figure 7. Liver held at 15,000 psi for 5 min shows little change. The paucity of cristae is typical of the mitochondria from sheep liver (×63,000).

fig.4

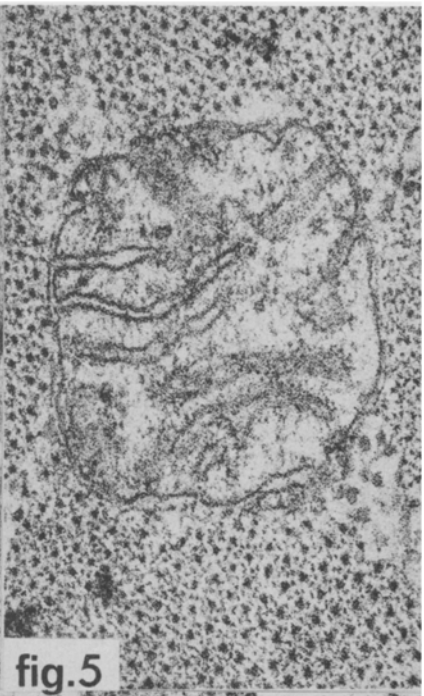
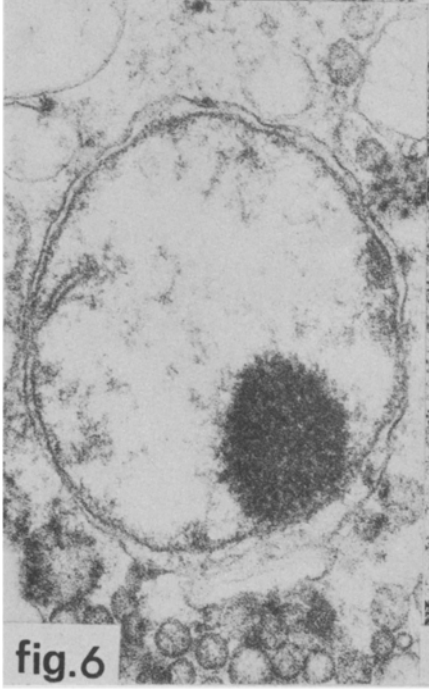


fig.5



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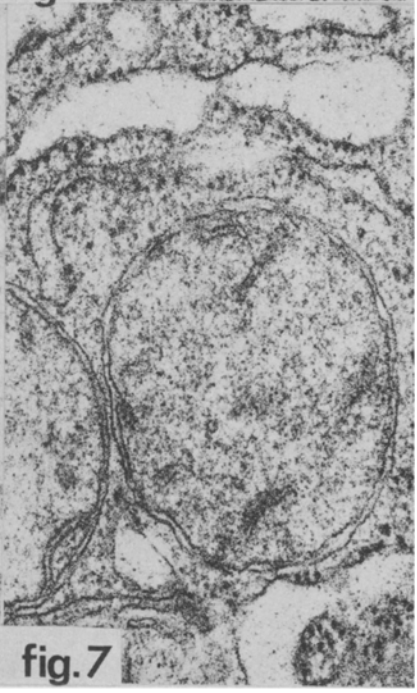

fig.7

the muscle [8]. In fact these intracristal structures may be seen occasionally in post rigor muscle, but this is generally limited by the gross swelling which also occurs (Rowe and Weidemann, unpublished observation). It is of interest that pressure has little effect on the pH of liver and does not induce the formation of intracristal structures.

Other conditions may also be imposed upon muscle by prolonged pressure treatment, which may contribute to the formation of these structures. Calcium ions normally present in muscle in high concentrations could conceivably contribute to the appearance of these intracristal structures. However, they bear no morphological similarity to the needle-like calcium crystals accumulated in cardiac muscle mitochondria when the muscle is soaked in a calcium enriched perfusion medium [9]. The matrix granules on the other hand do show some similarities to the granules accumulated in mitochondria after incubation with various ions [10]. The granules seen in isolated mitochondria incubated with calcium or magnesium ions were of about 20 nm diameter which is rather smaller than those seen here in muscle mitochondria and very considerably smaller than the 300-400 nm particles in the mitochondria of pressure treated liver. Porter [11] has shown, however, that with increasing accumulation of ions, the granules increase in size rather than in number. Mitochondrial granules similar to those presently described are found in ischaemic dog myocardium [16]. The presence of these granules in both liver and skeletal muscle mitochondria may therefore be due to anaerobic conditions during the pressure treatment.

At present no evidence is available as to the composition of either the intracristal structure or the matrix granules. A number of enzymes have been localized in the intracristal space of mitochondria of muscle including creatine kinase and adenylate kinase [12] and Sottocasa *et al.* [13] have presented evidence for the presence of a glycoprotein in the intermembrane space of rat liver mitochondria. On the other hand Suzuki and Mostofi [14] consider that the parallel filamentous intracristal inclusions in rat kidney mitochondria are probably derived from the cristal membrane and are probably lipid in composition. In the present case, it might be expected that if this is the origin of the intracristal material, it would also be seen in the compartment between the inner and outer mitochondrial membranes.

Apart from the known complement of matrix enzymes [12], evidence has been presented for the presence of a matrix skeleton in mitochondria which can only be visualized after critical-point drying [15]. This offers a further possibility for a source of material which could give rise to the matrix granules in both liver and muscle.

While the intracristal inclusions described in the present study show some differences in dimensions from the structures described by Hall and Crane [2, 3] they are similar enough to warrant the exercise of

considerable caution in the interpretation of their biological significance. It opens the possibility that intracristal structures can arise as a preparative artefact in those situations where there is extensive tissue manipulation.

Acknowledgements

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