

## INTRACRISTAL RODS

### A New Structure in Beef Heart Mitochondria

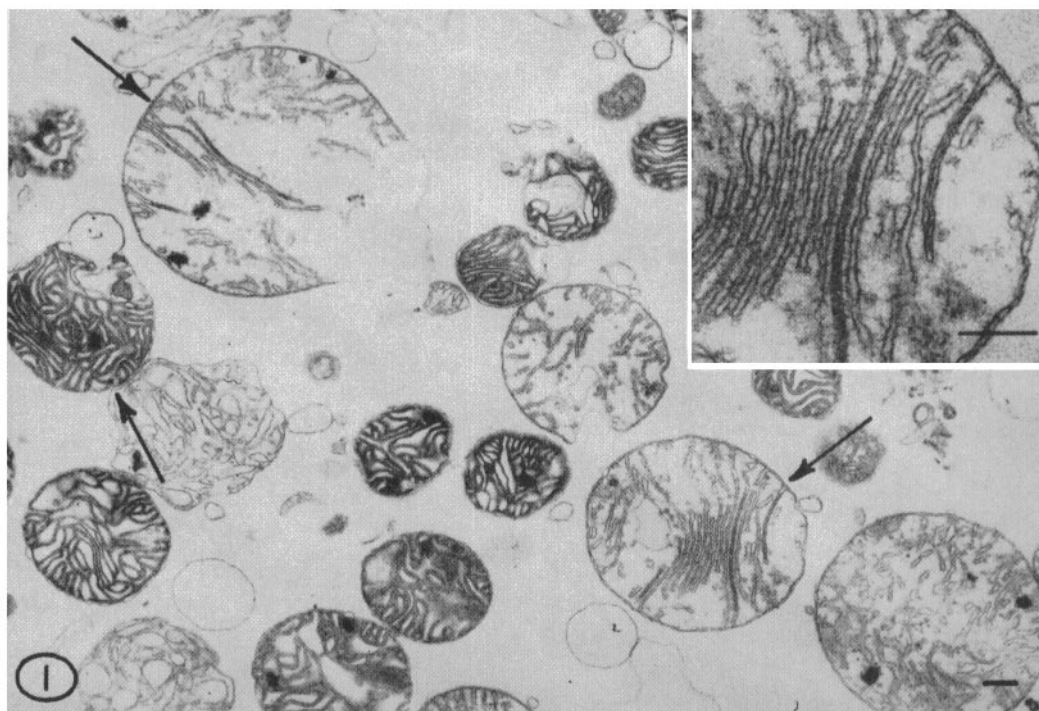
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Workers in many laboratories (1, 4, 5, 11, 13-15) have isolated outer membrane, inner membrane, and matrix fractions from beef heart and rat liver mitochondria and have assayed the enzymes associated with each fraction. Little attention has been directed towards the contents of the intracristal space, that space within the infoldings of the cristae and continuous with the space between the outer and inner membranes of the mitochondrion. Adenylate kinase, creatine kinase, and perhaps nucleosidediphosphate kinase are reportedly localized in this space (3, 6, 14), as these enzymes are eluted from mitochondria which have a

broken, but attached, outer membrane. However, no structural element has been reported in the intracristal space. A new structure which has been observed in the intracristal space of isolated heavy beef heart mitochondria by means of thin sectioning will be described here.

#### MATERIALS AND METHODS

Heavy beef heart mitochondria (2) were prepared after isolation by the method of Löw and Vallin (7). A mitochondrial suspension was fixed in one of the following: 4% glutaraldehyde, formaldehyde, or acrolein containing 0.1 M phosphate at pH 7.4 (2 hr);



Marker lines on all figures are equivalent to  $0.2 \mu$ .

FIGURE 1 Low magnification view of isolated heavy beef heart mitochondria. Three of the mitochondria in the field (arrows) have darkly staining cristae. Fixation in 4% glutaraldehyde; postfixation in 1%  $\text{OsO}_4$ . *Inset*, a higher magnification of one of these mitochondria showing the electron-opaque structure in the intracristal space. Fig. 1,  $\times 20,500$ . *Inset*,  $\times 50,000$ .

1% OsO<sub>4</sub> buffered at pH 7.4 with Veronal acetate (2 hr); 4% glutaraldehyde (1 hr) with postfixation in 1% OsO<sub>4</sub> (2 hr) and buffered as above; or 2% potassium permanganate containing Veronal acetate at pH 7.2 (2 hr). Specimens were dehydrated in an acetone series and embedded in Luft's Epon 812 (8). Silver and gray sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome and were mounted on copper grids. Sections were stained with 2% aqueous uranyl acetate and Reynold's lead citrate (12), and then examined in a Philips EM 300 microscope.

#### OBSERVATIONS

The examination of isolated heavy beef heart mitochondria at low magnifications following fixation in glutaraldehyde-OsO<sub>4</sub> has revealed that some mitochondria exhibit darkly staining cristae cristae (Fig. 1). Higher magnification (inset, Fig. 1) shows that these cristae contain an electron-opaque structure. As judged from the attachment of the crista to the inner membrane, the structure is clearly located in the intracristal space and not in the matrix. This intracristal structure occurs in mitochondria in the condensed as well as the orthodox configuration.

The shape and extent of the intracristal structures were determined by serial sectioning. In such a series, as represented in Fig. 2, the structures can be followed for some distance within a single crista without showing any change in size or shape.

The intracristal structure is not observed in all cristae or in all mitochondria. With glutaraldehyde-OsO<sub>4</sub> fixation the structure is seen in 20–30% of the mitochondria. The structure occurs in intact mitochondria which appear normal in all respects, as well as in swollen or ruptured mitochondria. Frequently, the intracristal structure is observed where a number of cristae align in a parallel fashion, as in Fig. 3. Since isolated mitochondria assume a roughly spherical configuration, no correlation can be made with respect to the original longitudinal axis of the mitochondrion.

The effect of other fixatives on the intracristal structure was investigated. With glutaraldehyde fixation (Fig. 4) the over-all preservation and appearance of the mitochondrion are different than with glutaraldehyde-OsO<sub>4</sub>. The cristal membranes are frequently apposed, as are the inner and outer membranes. Thus, the intracristal space and the space just under the outer membrane ap-

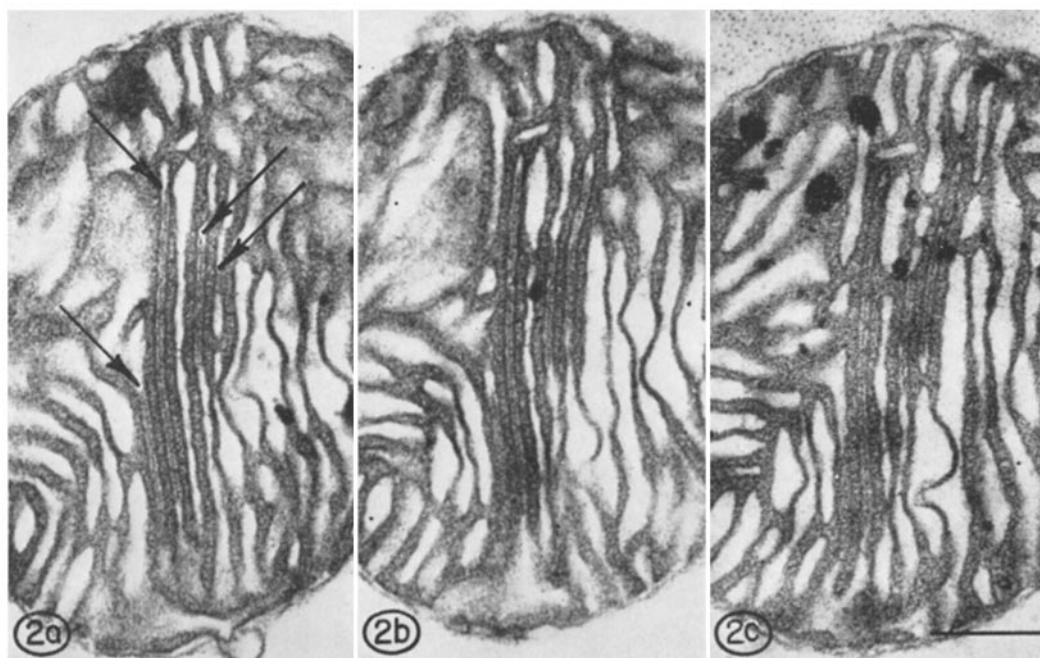
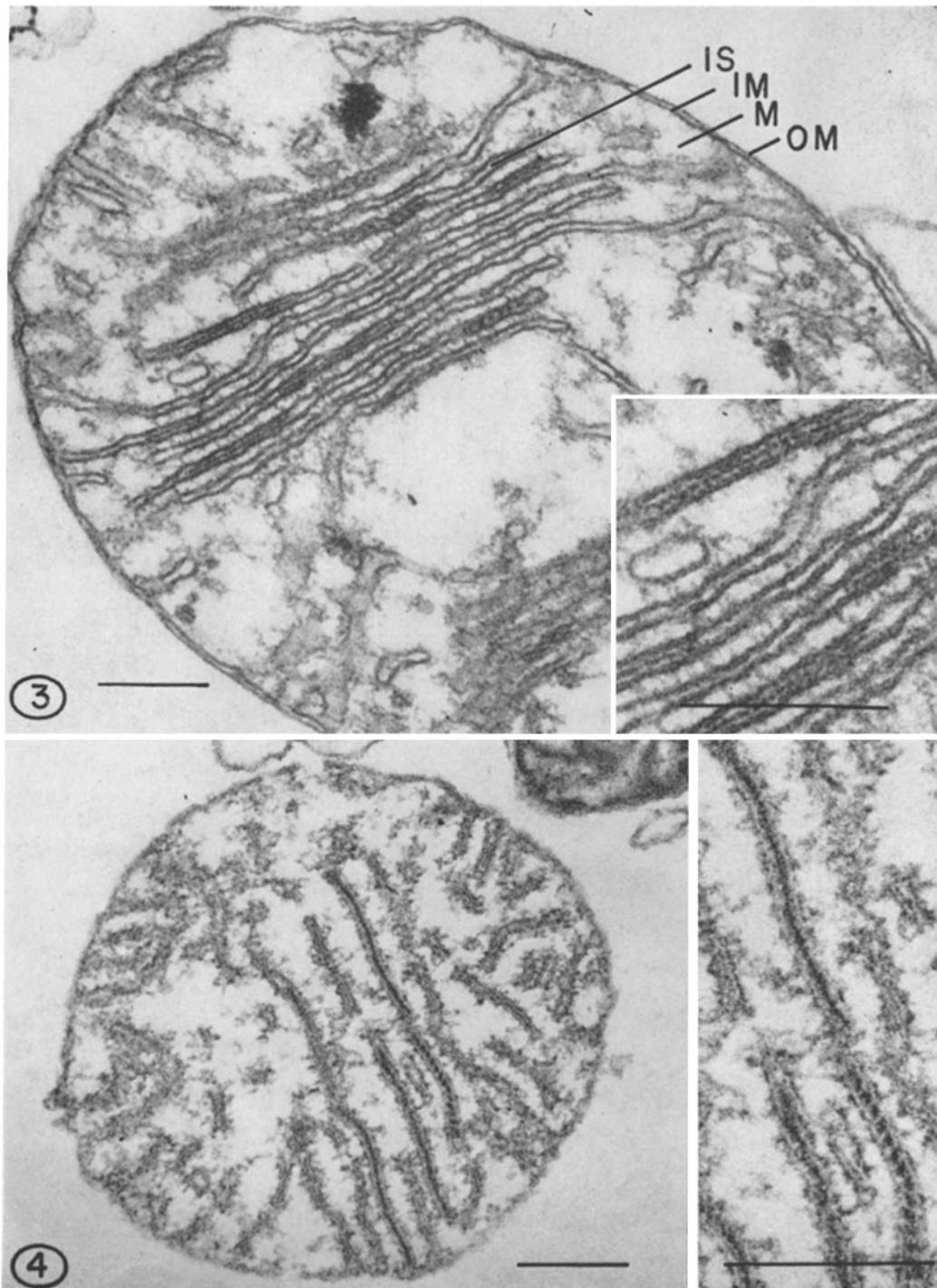
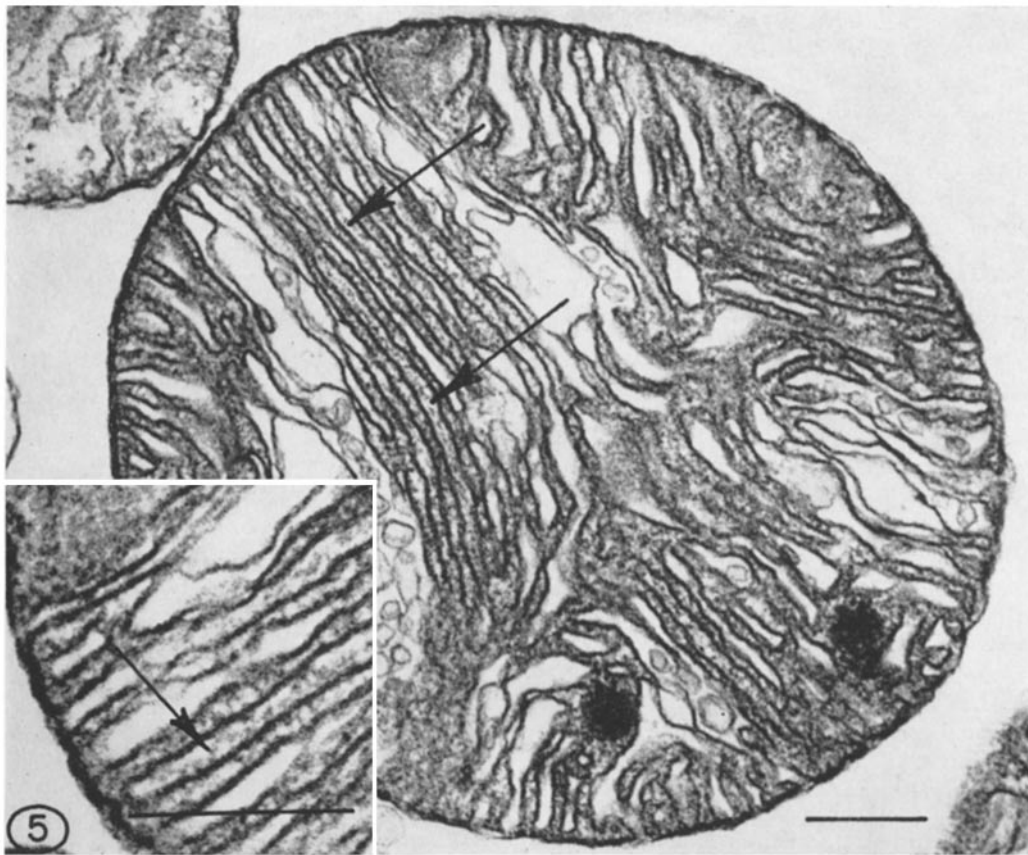


FIGURE 2 Sections through a condensed mitochondrion having four separate electron-opaque structures (arrows) in the intracristal space. The intracristal structures should not be confused with random, dense particles in the matrix. These are the second (2 a), third (2 b), and fourth (2 c) sections of five successive sections. Fixation in 4% glutaraldehyde; postfixation in 1% OsO<sub>4</sub>.  $\times 72,000$ .



**FIGURE 3** Mitochondrion fixed in 4% glutaraldehyde and postfixed in 1%  $\text{OsO}_4$  showing the intracristal structure with a slightly beaded appearance. *IS*, intracristal space; *IM*, inner membrane; *M*, matrix; *OM*, outer membrane. *Inset*, a higher magnification of the intracristal structure. Fig. 3,  $\times 80,000$ . *Inset*,  $\times 150,000$ .

**FIGURE 4** Mitochondrion fixed in 4% glutaraldehyde showing the intracristal structure both as a dense line and as a row of evenly spaced particles. *Inset*, a higher magnification of the intracristal structure. Fig. 4,  $\times 80,000$ . *Inset*,  $\times 150,000$ .



**FIGURE 5** Mitochondrion fixed in 1%  $\text{OsO}_4$ . The intracristal structure is not observed after this fixation procedure. Note the electron-opaque material (arrows) in the intracristal space. *Inset*, a higher magnification of the electron-opaque material. Fig. 5,  $\times 80,000$ . *Inset*,  $\times 150,000$ .

pear collapsed or missing. Even so, the intracristal structure is readily identified as an electron-opaque line squeezed between the two closely apposed cristal membranes.

Fixation of mitochondria in 4% formaldehyde or acrolein also reveals the intracristal structure. Extremely poor preservation of mitochondria is obtained with formaldehyde. With acrolein the mitochondria resemble those fixed in glutaraldehyde.

Mitochondria fixed only in  $\text{OsO}_4$  (Fig. 5) have a more swollen appearance than those fixed with any of the other fixatives used except formaldehyde. The outer membrane is frequently broken or lost, and the intracristal space is slightly swollen. No intracristal structure is observed. However, electron-opaque material is often present in the intracristal space. This material does not occur

uniformly throughout the entire space and could represent the unorganized or disorganized intracristal structure.

With potassium permanganate fixation, the intracristal space is free of any structure or electron-opaque material.

The intracristal structure occurs in a variety of forms and dimensions depending upon the plane of section as well as upon the fixative(s). It can appear as an electron-opaque line running the length of a crista and having electron-lucent regions on either side (Figs. 1-4). The line is about 60 Å wide with glutaraldehyde fixation and 45-50 Å wide with glutaraldehyde- $\text{OsO}_4$  fixation. The structure also appears as a row of evenly spaced particles (Fig. 4). The diameter of the electron-opaque particles is 75 Å in glutaraldehyde-fixed and 50 Å in glutaraldehyde- $\text{OsO}_4$ -fixed mitochon-

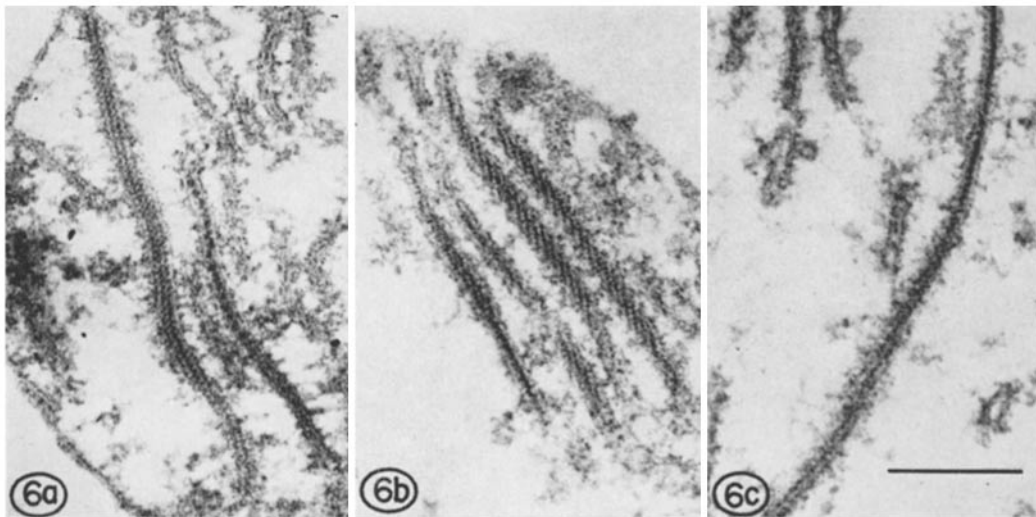


FIGURE 6 Mitochondria in which the cristae containing the intracristal structure have been sectioned obliquely. 6 *a*, Parallel lines run the length of the crista. 6 *b*, Parallel lines run obliquely to the length of the crista. 6 *c*, As the crista curves in the plane of the section the intracristal structure gives rise to a number of parallel oblique lines. Fixation in 4% glutaraldehyde.  $\times 91,000$ .

dria. Usually the particles are spherical but in some sections rows of ellipsoidal particles have been observed. There is no evidence that the intracristal structure is attached to the inner membrane of the mitochondrion at any point.

When a crista containing the intracristal structure is sectioned in an oblique rather than a normal plane, a pattern of parallel lines is observed (Fig. 6). The parallel lines may run the length of the crista or obliquely to the length of the crista. Occasionally, the intracristal structure is seen just under the outer mitochondrial membrane in that space between the inner and outer membranes.

#### DISCUSSION

The intracristal structure is observed in mitochondria that are fixed in an aldehyde fixative or in glutaraldehyde- $\text{OsO}_4$ . Fixation in  $\text{OsO}_4$  alone or in potassium permanganate appears to destroy the structure. This suggests that the structure is composed predominantly of protein.

Nevertheless, the possibility of an artifact arising from aldehyde fixation needs to be considered. Three facts support the reality of the structure. First, the intracristal structure is not seen in all cristae. A fixation artifact would be expected to occur uniformly throughout the intracristal space. Secondly, when the concentration of glutaraldehyde is varied from 0.25 to 4.0%, the frequency of

occurrence of the structure does not change. An artifact should increase in frequency as higher concentrations of the fixative are used. Lastly, three different aldehyde fixatives, glutaraldehyde, formaldehyde, and acrolein, reveal the same ultrastructural image of the structure. It is unlikely that these considerably different aldehyde fixatives would produce the same artifact.

Since phosphate buffer is used with all the aldehyde fixatives, it too must be eliminated as a source of artifact. Intracristal structures are observed in mitochondria fixed in cacodylate-buffered glutaraldehyde. Likewise, substitution of the Tris buffer used in the preparation of the heavy mitochondria did not alter the occurrence of the structure.

The variation in the appearance of the intracristal structures can be explained by differences in the plane of the section if the structure exists as an array of parallel rods within the intracristal space. Hence, the name "intracristal rods" seems appropriate. When the plane of section is parallel to the length of the rods and normal to the crista, a dense continuous line is seen within the intracristal space (Figs. 1-4). If the plane of section is normal to the length of the rods as well as to the crista, a row of spherical particles is observed (Fig. 4). Each sphere represents a cross-section of a single rod. If the rods are sectioned obliquely

but the plane of section is normal to the crista, a row of ellipsoidal particles is seen. If both the rods and the crista are sectioned obliquely, a number of parallel lines are observed (Fig. 6).

A structure similar to intracristal rods has been reported as an atypical crista in bean root mitochondria (10), and compared to the tight junctions seen in animal cells. The intracristal rods observed in heart mitochondria clearly do not resemble a tight junction, for two reasons. First, typical cristal membranes of usual dimensions appear on either side of the intracristal rods and are continuous with cristal membranes where the rods are not present. Secondly, the intracristal rods remain when the adjacent cristal membranes diverge.

Mollenhauer et al. (9) have described an intracristal structure in the Golgi apparatus of *Euglena* which resembles intracristal rods in several respects. With glutaraldehyde-OsO<sub>4</sub> fixation the intracristal structure appears as a dense line within the lumen of the cisterna midway between the bounding membranes. Intracristal rods occupy a comparable position within the intracristal space. Both the intracristal and the intracristal structures are structurally different from, and not continuous with, the bounding cisternae or cristal membranes.

The nature and function of intracristal rods have not been determined. The structure may represent an enzyme or enzyme complex, a precipitated storage product, or a purely structural element. Further work is necessary to elucidate the functional significance of intracristal rods.

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