OBSERVATIONS ON MITOCHONDRIAL STRUCTURE

Angular Configurations of the Cristae

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ABSTRACT

This paper reports the common occurrence of creases or sharp ridges on the membranes of the mitochondrial cristae. In section these appear as sharp angulations recurring at more or less regular intervals along the profile of the crista. In instances where such angulations occur alternately on one membrane and then on the other, the crista has a zig-zag course. Where they occur at the end of a crista its profile has a square tip. An exaggerated expression of this tendency for angulation of the internal mitochondrial membranes is found in certain bat muscles where some of the cristae take the form of parallel bundles of prismatic tubules which are triangular in cross-section. Angular configurations of the cristae have been observed after various methods of specimen preparation, in a variety of cell types, and in a wide range of animal species. They are believed to be a normal variation of the basic structural organization of the mitochondrion and to be the morphological expression of a property which the internal mitochondrial membranes do not share with other membranes in the cell.

Since the discovery by Palade (1) of a common basic plan of internal organization in the mitochondria of many different cell types, investigators have been more interested in probing the generality and significance of this master plan than in documenting minor differences in the mitochondria of particular cells or particular animal species. Variations in shape, orientation, and number of cristae, as well as in the density of the matrix and the abundance of mitochondrial granules have, however, been recognized from the outset (1), and evidence is steadily accumulating which indicates that significant biochemical differences are associated with these variations (2, 3). The time may not be too far distant when we will be able to correlate particular structural configurations with specific biochemical properties of mitochondria in different cell types (4).

In diagrammatic representations of the typical mitochondrion, the outer limiting membrane is smooth contoured and continuous whereas the innermost is plicated to form the thin folds or incomplete septa that constitute the cristae. The two leaves of a crista are usually depicted as running straight and parallel, separated by a narrow interspace of uniform width. When minor departures from this idealized form have been observed in electron micrographs, they have usually been considered either random variations of no significance or artifacts of specimen preparation. As improved methods of embedding have brought greater reproducibility of preservation, some of these minor irregularities in the contour of the cristae have disappeared but, in our experience, one irregularity has persisted with sufficient frequency to warrant the conclusion that it represents a significant general feature of mitochondrial structure worth drawing to the attention of other investigators. We refer to a tendency to abrupt angulation of first one and then the other of the membranes of a crista at more or less regular intervals along its length. This configuration of the cristae has now been observed in mitochondria of numerous cell types in animal species ranging from invertebrates to the higher mammals. It is reported here in the belief that this peculiar angulation may reflect a periodic discontinuity of structural organization of the membranes at the molecular level. This may prove to be of interest in relation to the emerging biochemical concept of the ultrastructure of the mitochondrion, which envisions units or assemblies of enzymes arranged in particular topographical relation.

MATERIALS AND METHODS

The observations on mitochondrial structure reported here were incidental to other studies on a variety of organs in a considerable number of animal species. Those tissues which have been examined with more than passing interest include the cricothyroid muscles and pancreas of the bats *Myotis lucifugus* and *Eptesicus fuscus;* the gastric mucosa of the frog, *Rana pipiens;* the so called chloride cells of the gills of the euryhaline fish, *Fundulus heteroclitus*; the liver of the plethodontid salamander, *Batrachoseps attenuatus*; and the nervous system of the medicinal leech, *Hirudo medicinalis.* Although the micrographs studied are from the work of several different investigators, the methods of specimen preparation were quite similar and involved use of collidine or Veronal buffered osmium tetroxide fixation (pH 7.0–7.4), rapid dehydration, embedding in Epon and lead staining by the methods of Karnovsky (5) or Millonig (6).

OBSERVATIONS

In contrast to the perfect parallelism usually depicted for the two membranes that constitute a crista, one membrane is often seen to diverge slightly from the other, then to form a clearly defined angle of 90° or more that brings it back to normal distance from the other leaf of the crista which has continued undeflected or only slightly curved with its convexity toward the angulation of the first membrane. A short distance farther along the same crista the other membrane may form a similar sharp angle on the opposite side. This tendency for the angulation of the two membranes to alternate in direction may impart a zig-zag course to the cristae (Figs. 1 and 2). Not infrequently, adjacent cristae are angulated in the same direction at corresponding intervals along their length and thus follow a parallel zig-zag course (Figs. 1 and 4).

Although alternation in direction of angulation is common, it is by no means invariable. The same side of the crista may show two or more successive angles with no corresponding deflections of the other membrane. This is particularly common in mitochondria in the neurones of the leech (Fig. 3) where the cristae are longer than the diameter of the organelle and therefore tend to form concentric loops. Here the membrane on the outer, convex surface of the loop may show several sharp angles while the inner membrane has a smooth, curving contour. At the free edge of a crista where its two membranes are continuous, two such angulations occurring close together may result in cristae which appear in section to have square ends.

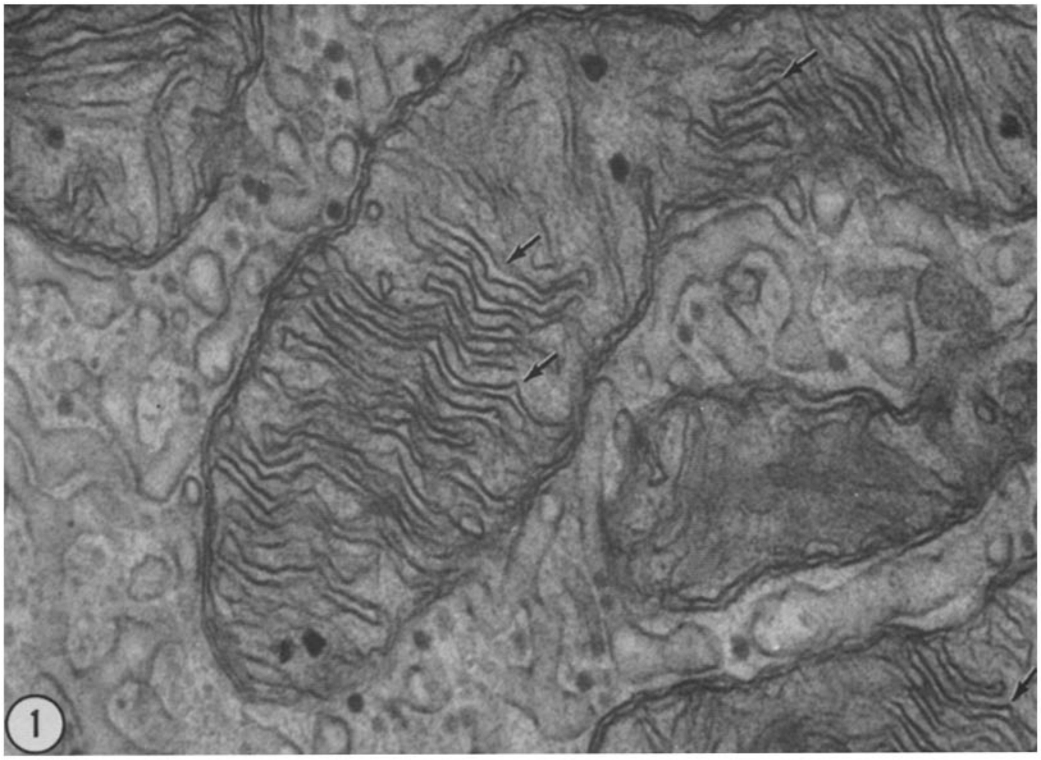
The angular configuration of the cristae described above is quite common. It has doubtless been seen by other investigators but was passed off as an accident of specimen preparation resulting, in some way, from mutual deformation of the membranes during dehydration or as one of the vagaries of the particular method of embedding. Upon reflection, however, it is difficult indeed to account for such a form on the basis of compression, shrinkage, or swelling, which are the

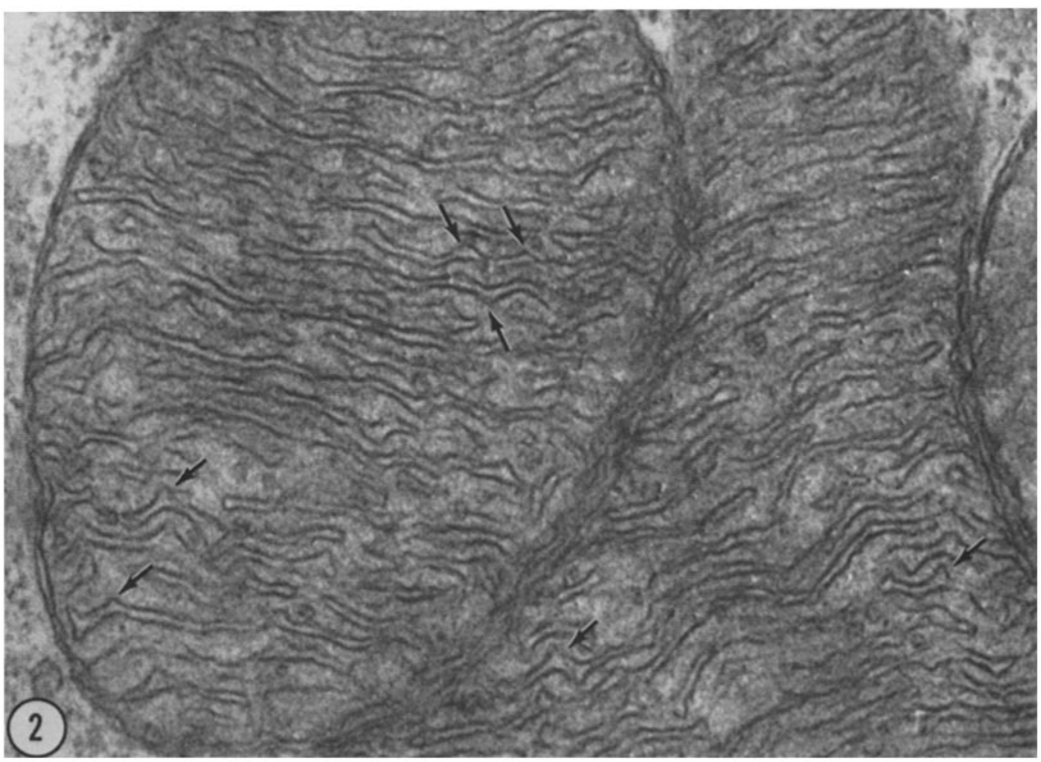
FIGURE 1

Mitochondria from a "chloride cell" of the gill of *Fundulus.* The cristae show a marked tendency to angulation of first one leaf of the crista and then the other. The arrows point to a few of the numerous examples of angular profiles on the cristae of the mitochondria in this field. × 70,000.

FIGURE 2

Mitochondria from the cricothyroid muscle of *Myotis.* The cristae are long and very numerous in this fast acting muscle. At intervals along their length each of the pair of membranes forming a crista makes sharp angles, some of which are indicated by the arrows. The tendency for alternation of these angles on one side and the other imparts to the crista a slight zig-zag course. × 60,000.





factors usually invoked to explain histological artifacts. Our own experience is mainly with Epon-embedded material processed by the methods which have become routine in this laboratory. We have seen similar cristae, albeit more rarely, in methacrylate-embedded material and have identified them also in published micrographs of other investigators using Vestopal. A striking example is to be found in the very high magnification electron micrograph which appeared on the cover of *Science*, showing a mitochondrion prepared by Dr. Fernández-Morán, using the liquid helium freezing method (7). It can be inferred, therefore, that the phenomenon described here is not the product of any one method of specimen preparation. Although it is reasonable to suppose that this tendency to angulation may be exaggerated by some methods and partially obscured by others, it would seem incontestable that there is an underlying basis for it in the molecular organization of the membranes of the cristae. Whatever this may be, it does not seem to be a property that is shared with the outer limiting membrane of the mitochondrion or with any of the other membranes of the cytoplasm.

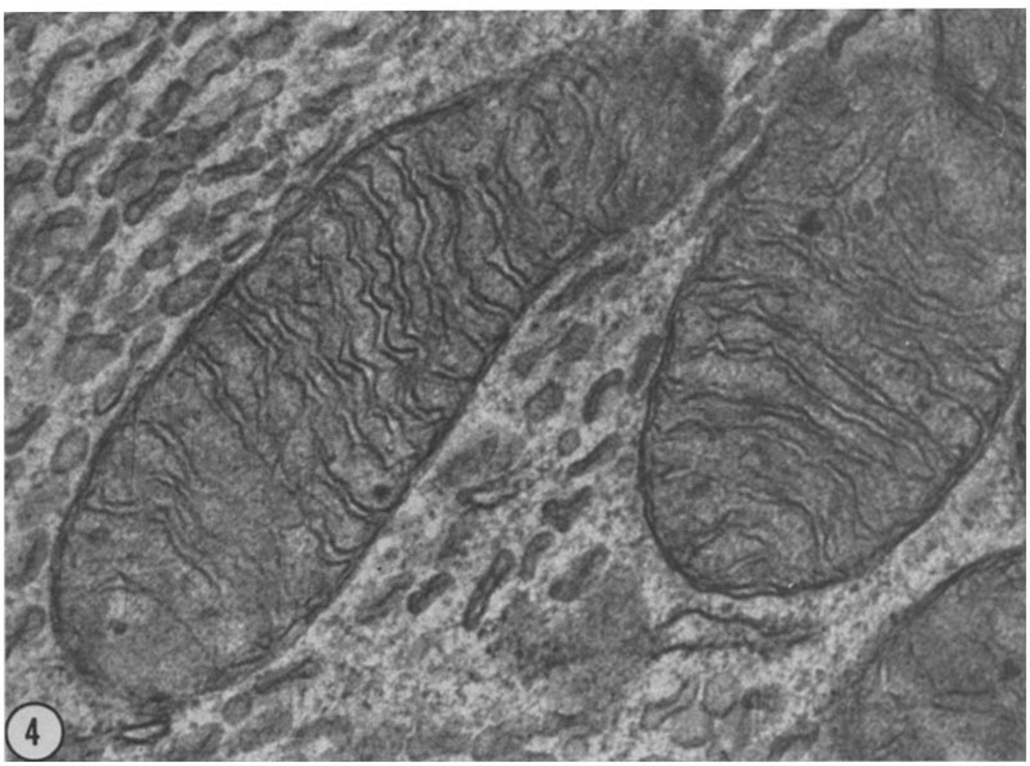
Another observation, which has given us confidence in the reality of the angular profiles commonly seen in mitochondrial cristae, is the occurrence of an extreme expression of this same tendency in the mitochondria of the cricothyroid muscle of the bat larynx. The mitochondria in this material are located in rows between myofibrils and in large clusters in the peripheral sarcoplasm immediately beneath the sarcolemma (8). Mitochondria 5 μ or more in length are not uncommon in this material, and they average about 0.5 μ in width. The cristae are very abundant and usually take the form of closely packed parallel septa that extend the full width of the organelle and are perforated by occasional circular fenestrations. In addition to cristae of this kind, triangular profiles are encountered in many of the mitochondria. These equilateral trigonal figures may occur singly but are more often found in groups of six arranged radially with their sides parallel so that they form a more or less regular figure (Figs. 7, 9, and 10). The sides of the triangles are about 600 A long and are sometimes slightly concave. In mitochondria sectioned in other planes, it is evident that the triangles are cross-sections of prismatic tubules (Figs. 5, 8, and 11). These present a pattern of parallel stripes when cut longitudinally (Fig. 8, M_2). The width of the stripes varies depending upon the level at which each prismatic tubule in the group is sectioned. The patterns obtained when the plane of section deviates from true longitudinal or transverse (Fig. 10, Fig. 8, M_3) become complicated and more difficult to interpret. Although the fascicles of prismatic tubules usually run quite straight, they may bend abruptly at approximately a right angle (Fig. 11). Thus in some instances, the longitudinal or oblique pattern of parallel stripes can be seen at one end and the triangular cross-sections at the other end of the same mitochondrion. The triangular prismatic cristae usually occupy a sharply localized area of the mitochondrion and are completely surrounded by foliate cristae of the usual kind. End-to-end continuity of the one with the other can often be demonstrated (Fig. 8, M_2 at arrow). Moreover, one can identify forms transitional between the angulations on the lateral surfaces or ends of the foliate cristae, described above, and the tubular prismatic cristae (Fig. 8, M_1 at arrow; Fig. 6, at arrows). It seems clear, therefore, that the latter

FIGURE 3

An area of the neuropil from the central nervous system of *Hirudo* showing parts of three axons surrounded by glial cytoplasm rich in fine filaments. Two of the mitochondria in the axons have long cristae that form concentric loops. Here the membrane on the outer convex surface of the loops shows several sharp angles (arrows) while the inner membrane has a smooth-curving contour. × 60,000.

FIGURE 4

Two mitochondria from a secretory cell in the gastric mucosa of *Rana*. Several of the cristae in the mitochondrion at the left of the figure show a parallel zig-zag course due to the fact that their membranes are angulated in the same direction at corresponding intervals along their length. × 70,000.



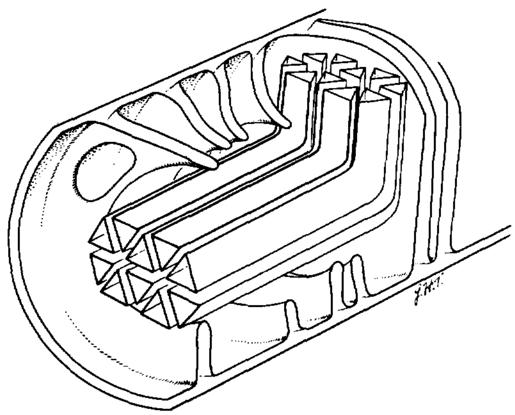


FIGURE 5

Diagrammatic representation of a mitochondrion from the cricothyroid muscle of the bat showing hexagonal packing of a group of prismatic tubules. These make a right angle bend. The bundle of prismatic cristae is surrounded by cristae of usual structure.

develop from the former and are simply another and more conspicuous expression of the same peculiar property of the mitochondrial membranes.

A change in direction of cellular membranes is usually accomplished in a gradual curve or a smooth-contoured fold. Abrupt changes of direction or sharp corners are very rare indeed. No suggestion can be offered at present as to the possible significance of the angular structure of mitochondrial cristae described here. It is as though the membranes of the cristae were composed of relatively inflexible segments or plaques joined to each other by more pliable junctional regions. The occurrence of images, suggesting that such a structure is present in a wide variety of cell types and in a considerable number of animal species, made it seem worthwhile to record this phenomenon in the hope that it may stimulate further investigations by others better equipped to explain its molecular basis or to relate it to the enzymatic topology of the mitochondrion.[1] In the case of the unusual triangular cristae in mitochondria of the bat cricothyroid, speculation as to their functional significance would not be profitable at present. This muscle is believed to have unusual physiological properties but the only function of the mitochondria, as far as we are now aware, is to provide energy for contraction and there is no reason to believe that prismatic cristae would confer any advantage in the exercise of this function. One might be more willing to speculate along this line if such structures were present in all of the mitochondria of this muscle, but, as in the examples of peculiar mitochondria that have been reported by others (9–14), the atypical structure is found in only a small fraction of the entire mitochondrial population (2).

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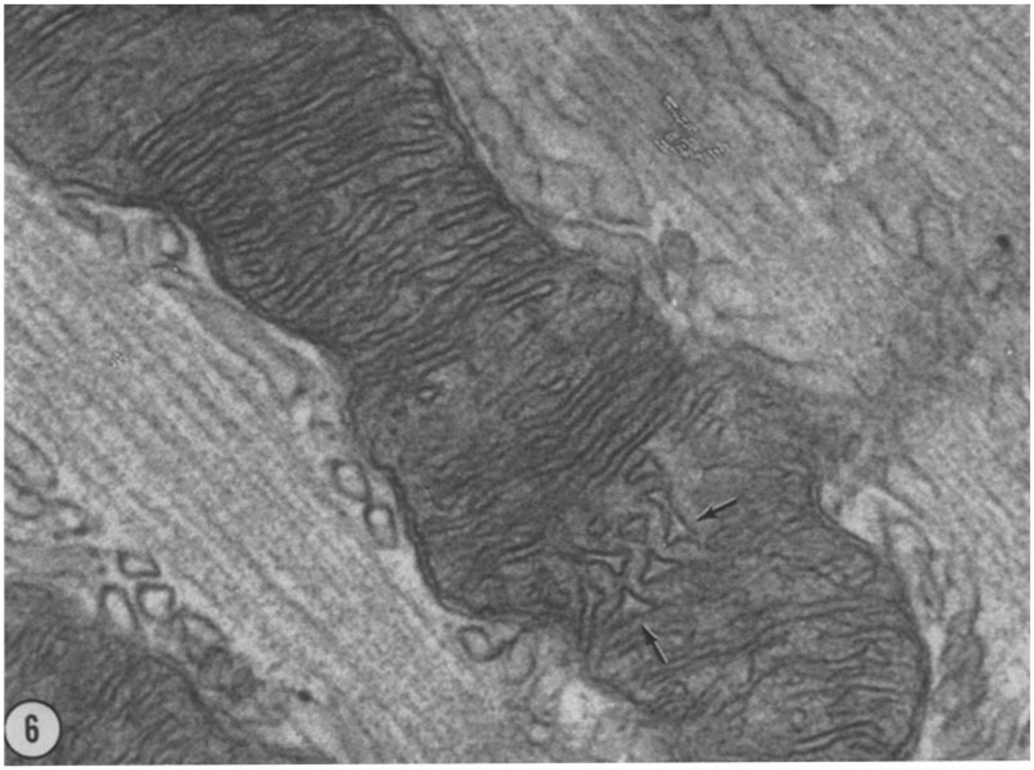
[1] If further justification for publication of this article were needed, it can be found in a paper just published by Luft *et al.*, (15) which reports a human case of severe hypermetabolism apparently caused by a defect in mitochondrial enzyme organization. A variety of structural alterations of mitochondria in muscle biopsies are described and these are believed to be related to the biochemical abnormalities found. Among those illustrated is a mitochondrion showing striking examples of zig-zag cristae. The authors might have been less likely to interpret this particular configuration of the cristae as a lesion correlated with the observed biochemical abnormalities had they been aware of the common occurrence of such angulations in the mitochondria of normal animals.

FIGURE 6

A mitochondrion in the cricothyroid muscle of the bat. The very numerous cristae are, for the most part, simple bilaminar folds of the inner limiting membrane. In the region indicated by the arrows, certain of the cristae show marked angulations of their membranes that appear to be transitional forms between typical cristae and the prismatic tubules that are found in some of the mitochondria of this muscle. × 70,000.

FIGURE 7

Mitochondria from the cricothyroid muscle of the bat. Most of the cristae are of the usual type but in the area indicated by the arrows there is a group of prismatic cristae with their triangular cross-sections uniformly spaced in a precise pattern. × 75,000.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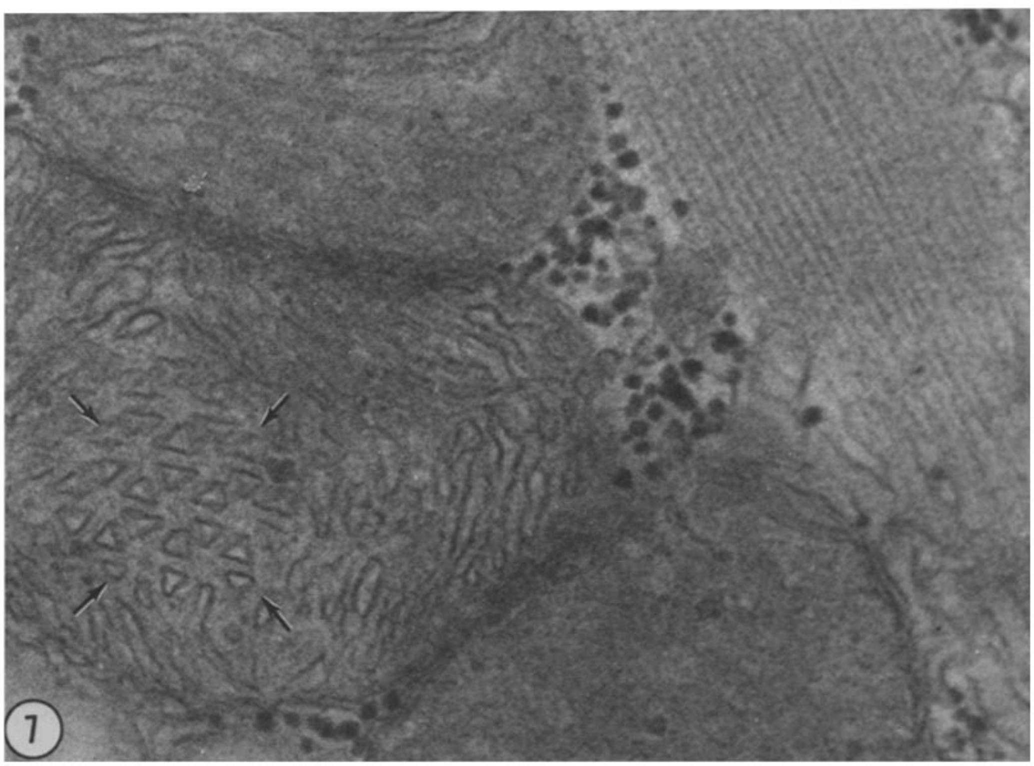
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Figure 8

A small area of a bat cricothyroid muscle fiber cut longitudinally, showing mitochondria lipid droplets (*Lp*) and membranous elements of the sarcoplasmic reticulum (*Sr*) in the clefts between the myofibrils (*Mf*). Three of the mitochondria (M_1, M_2, M_3) show prismatic cristae cut in different planes. One of these mitochondria (M_2) shows a fascicle of such prismatic tubules cut longitudinally and illustrates (arrow) the continuity of these with the ordinary cristae. The area indicated in the uppermost of these mitochondria (M_1) shows transitional forms between ordinary cristae and those having triangular cross-sections. In the mitochondrion at the lower edge of the figure, the prismatic cristae have been cut obliquely and present a confused picture. × 30,000.

Figure 9

Two mitochondria in the bat cricothyroid muscle. In one of these, only angulation of otherwise normal cristae is seen. In the other, there is a group of prismatic tubules cut in cross-section. × 45,000.

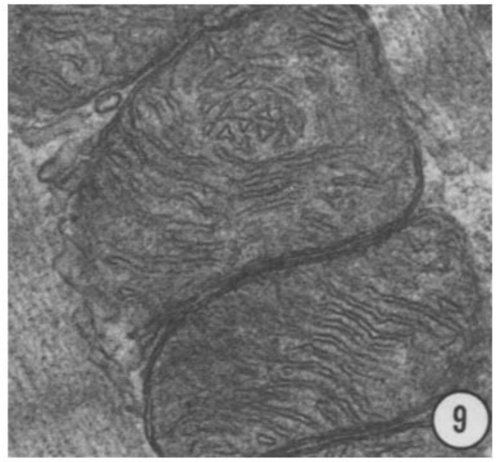
Figure 10

A mitochondrion close to the sarcolemma (*Slm*) showing an area of transversely and obliquely sectioned prismatic cristae. At the arrow, one member of an hexagonal array of such cristae still appears to be an angulated region of an ordinary crista which has not separated off to become an independent prismatic tubular element. × 45,000.

Figure 11

Portions of four mitochondria from bat cricothyroid muscle. At the arrow above, a group of prismatic cristae has been sectioned transversely. At the lower arrow is a bundle of similar cristae cut longitudinally. To the left of the arrow these cristae make an abrupt change of direction forming nearly a right angle. There are alternating dark and light stripes in the longitudinal section of the prismatic cristae. The dark stripe is observed when the section includes the base of one of the prisms. The density is caused by the limiting membrane of the crista seen in planar view. The fine line which splits the clear stripe into halves represents the apex of a prismatic crista. × 50,000.


Sr
Lp
M1
Mf
M2
M3
8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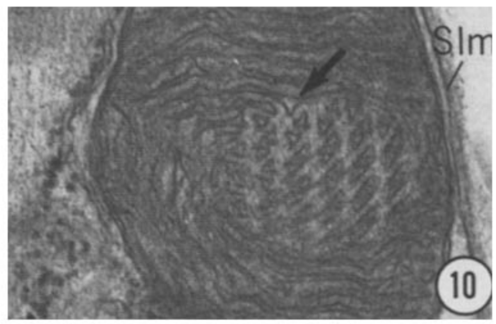
Slm
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