MORPHOMETRY AND ULTRASTRUCTURE OF PRISMATIC CRISTAE IN MITOCHONDRIA OF A CRAYFISH MUSCLE

A Hypothesis of the Structural Principle

L. L. MURDOCK, MARY ANNE CAHILL, and A. REITH. From the Fachbereich Biologie, Universität Konstanz, Konstanz, West Germany and Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo 3, Norway. Dr. Murdock's present address is the Waisman Center on Mental Retardation and Human Development, Madison, Wisconsin 53706.

Mitochondria with prismatic cristae have been observed in certain cell types from vertebrates (1, 2, 7, 8, 10), cyclostomes (14), and mollusks (3). The present study establishes that such mitochondria also occur in a representative of the phylum Arthropoda, specifically in the sphincter muscle of the vas deferens of the crayfish, *Astacus leptodactylus* Eschscholz and *A. astacus* L. We present morphometric and ultrastructural findings relevant to a description of the structure of these mitochondria and propose a hypothesis of the factors which lead to the formation of the geometric arrangement of cristal membranes.

MATERIALS AND METHODS

Adult *A. astacus* and *A. leptodactylus* were brought from commercial suppliers and maintained in charcoal-filtered running tap water at 9°C. Tissues were prepared during the months November to April. Vasa deferentia with attached sphincter muscles were dissected into crayfish saline (12) and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.25) for 1 h at room temperature (ca. 22°C) followed by 1 h in ice-cold 1-1.25% osmium tetroxide in 0.06 M phosphate buffer containing 0.2 M NaCl. All specimens were dehydrated in alcohol and embedded in Epon (6). Sections were stained in separate solutions of uranyl acetate (6% in 1% acetic acid) and lead citrate (9) and examined in either a Zeiss EM9s or an Elmiskop 1A electron microscope.

Cross sections of muscle fibers were used for stereological analysis. The volumetric composition of 30 fibers was determined by a lattice test system (13) at a final magnification of × 15,000. The test lines were spaced d = 0.27 μm. For the surface-to-volume ratios of mitochondria, a lattice test system spacing d = 0.04 μm was applied at a final magnification of × 85,000.

RESULTS

During the winter the crayfish vas deferens, which is a long (up to 40 cm in large *A. leptodactylus*)

tubular organ with a diameter up to 2 mm, is filled with viscous white seminal paste. At the distal end of the vas, there is a sphincter muscle which presumably serves as a valve regulating the release of seminal paste during copulation. The sphincter muscle is made up of several outer layers of circular muscle fibers and a few inner layers of longitudinally-oriented fibers. The number of mitochondria in the circular fibers is moderate, and they appear to be randomly distributed. The majority of them are elongate, contain septate or foliate cristae, exhibit a fairly electron-dense matrix, and do not differ in any marked way from those which are generally accepted as typical mitochondria (8). Scattered among this population of mitochondria is a second type, referred to here as "prismatic-type" or "angular-type" mitochondria (Figs. 1 and 2). In cross section the cristae of these mitochondria have either triangular or rhomboid profiles. Such profiles are surrounded by an array of electron-dense dots which appear to represent the two-dimensional expression of filaments as seen in longitudinal (Figs. 3 and 4) and serial sections (Fig. 5). The dots measure ca. 7 nm in diameter; their arrangement is not always perfectly regular but it does closely resemble the hexagonal pattern described by Morales and Duncan (7). Less frequently, regions of dot arrays occur with no cristae among them (Fig. 2). The mean spacing of the dots in this case (13.4 ± 3.2 nm, $n = 11$) is not significantly different from that in mitochondria containing abundant angular cristae (12.3 ± 1.5 nm, $n = 100$). The angular cristae with surrounding dot array may nearly fill the mitochondrion at some levels of section, but most frequently they occupy only the central region. A peripheral zone of normal-appearing matrix and cristae is usually seen.
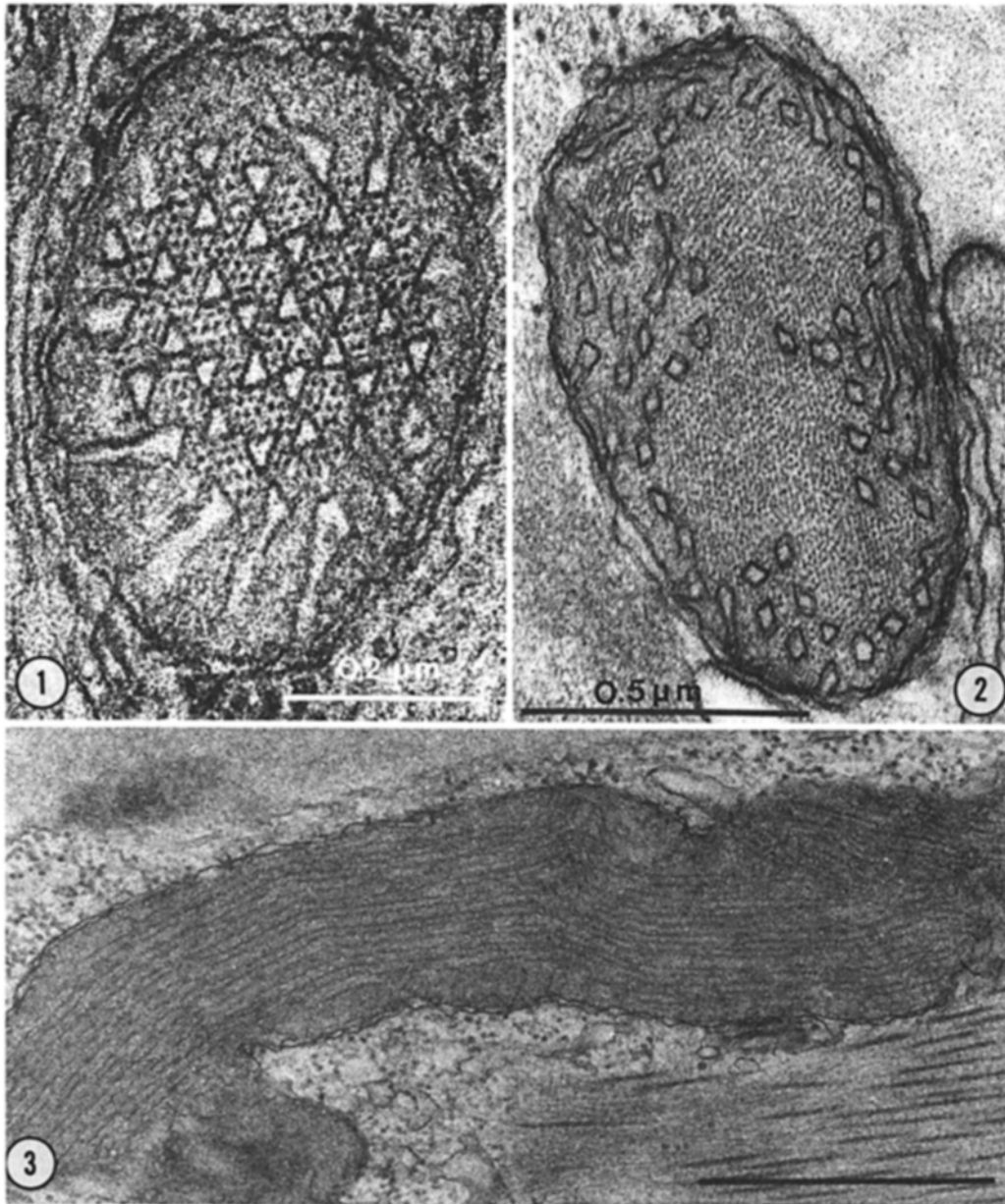
FIGURE 1 Cross-section of a mitochondrion located at the outer edge of a circular muscle fiber of the sphincter muscle of the crayfish vas deferens. Outer and inner mitochondrial membranes are easily distinguished. Invaginations of the inner membrane penetrating into the dot array in the central region assume angular profiles. Within the dot array are triangular profiles which are the two-dimensional expression of prismatic cristae which course through the long axis of the mitochondrion. × 135,000.

FIGURE 2 A mitochondrion exhibiting a large dot-array field and only scattered angular cristal profiles. The mean interdot distance in the large dot field does not differ significantly from that in mitochondria containing regularly ordered cristal profiles (see text). × 78,000.

FIGURE 3 Longitudinal section through a mitochondrion in a circular muscle fiber. Thick parallel lines course along the length of the mitochondrion. Thin lines running between and parallel to these thick lines can also be recognized at some points. The thick parallel lines are believed to represent longitudinal sections through the prismatic cristae. The thinner lines may be the longitudinal expression of the dot arrays seen in cross section; thus, the dots are actually the expression of filaments which run parallel to the prisms (see also Fig. 4 and text). Bar, 1.0 μm. × 40,000.
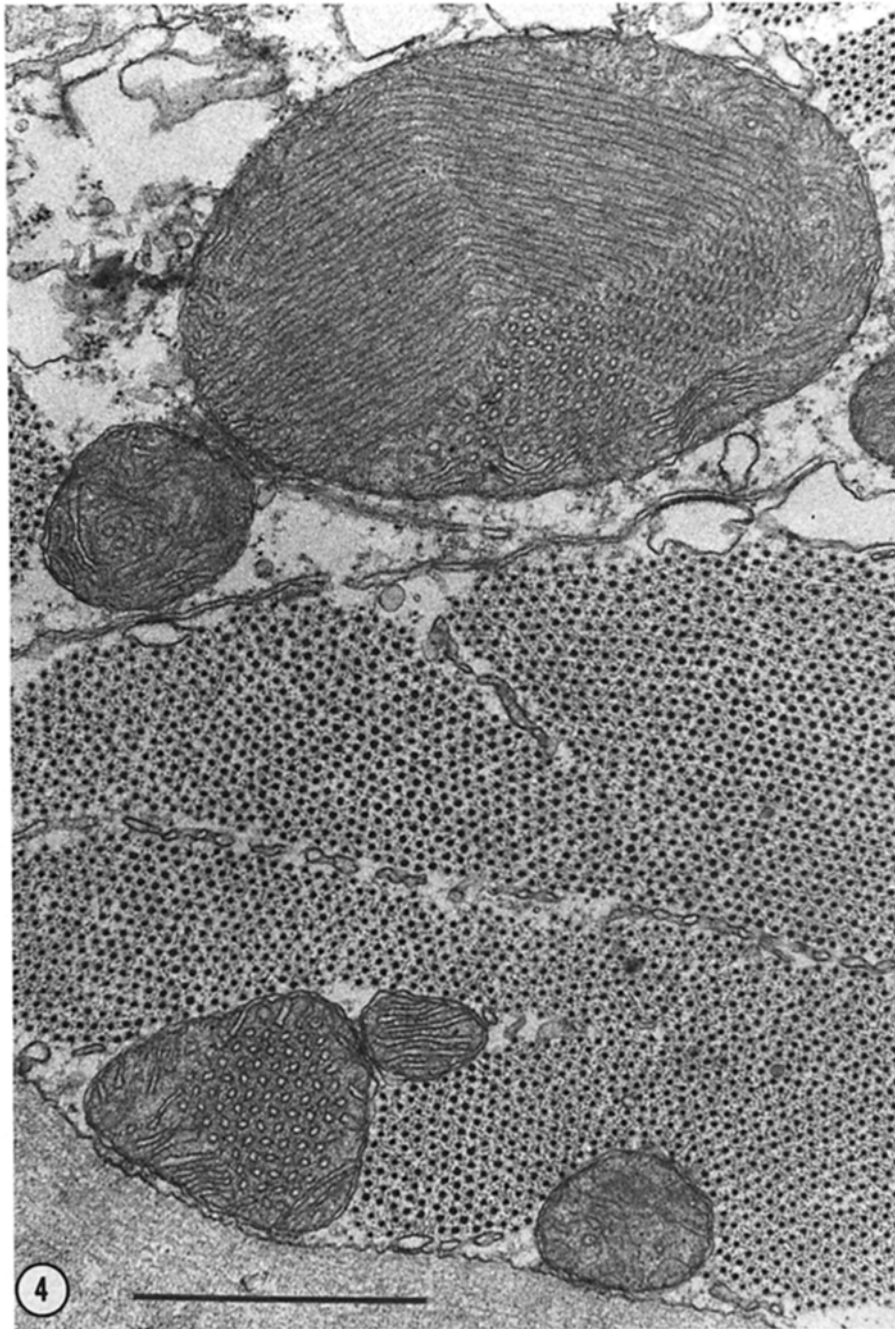
327

FIGURE 4 Combined cross and longitudinal section of a single mitochondrion exhibiting both the pattern of parallel lines described in Fig. 3 and angular profiles as in Figs. 1 and 2. It is evident that angular profiles and parallel lines represent different expressions of the same structure, depending upon the plane of the section. A second mitochondrion is shown which closely resembles that of Fig. 1, except that the cristal profiles in the center of the mitochondrion are diamond shaped. Adjoined to this is a third mitochondrion of the ordinary foliate type. Bar, 1.2 µm. × 32,000.
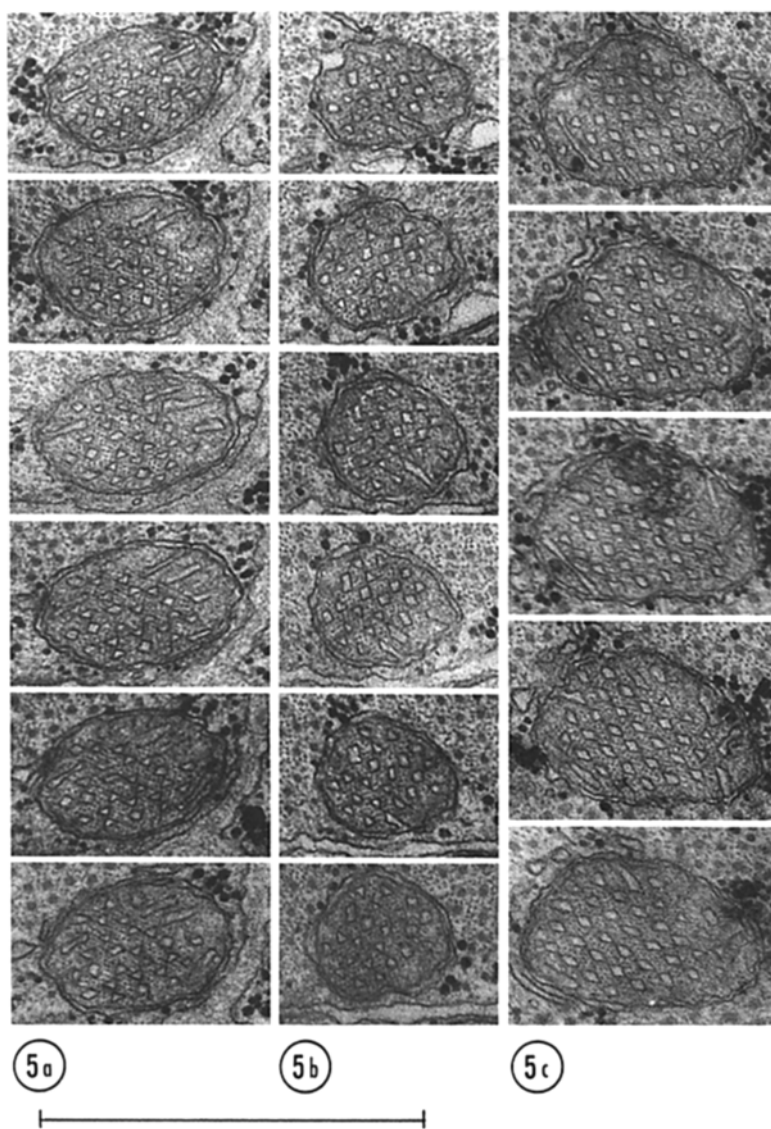
FIGURE 5 Serial cross-sections through a mitochondrion showing diamond and mixed diamond-shaped and triangular cristal profiles. It is clear that the profiles result from the presence of parallelopipeds or prisms which pass along the length of the mitochondrion. Likewise, the dot arrays probably represent the two-dimensional expression of filaments running parallel to the parallelopipeds. Near the periphery of these mitochondria, cross-sections of one or more foliate cristae can be seen in every section, showing that this type of crista also passes longitudinally along the length of the mitochondrion. That these foliate cristae are formed by invaginations of the inner membrane is particularly clear from Fig. 5b. Bar, 1.0 μm × 50,000.

In longitudinal sections the mitochondria exhibit both thick and thin parallel lines (Fig. 3). That these lines represent the edges of cristae and filaments is clear from occasional sections (Fig. 4) in which both parallel lines and cross-sections of prismatic cristae with dot arrays are seen to form a continuum. Further evidence that dot arrays and triangles represent continuous longitudinal structures is that their profiles are continuous in serial sections (Fig. 5). The serial sections also indicate
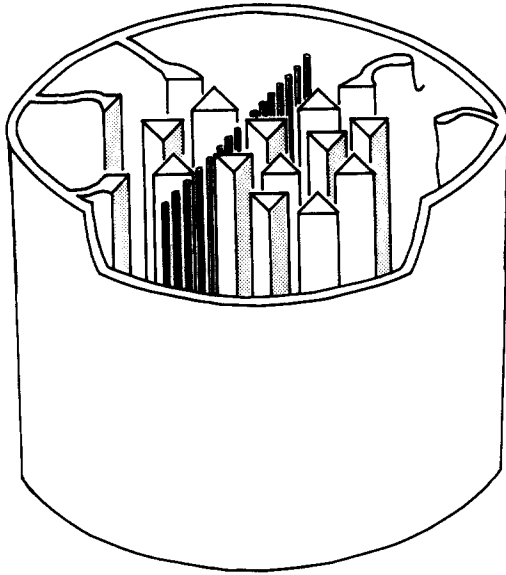
FIGURE 6 Three-dimensional diagrammatic representation of a mitochondrion containing prismatic cristae. For clarity, the hypothetical rodlets are here represented only by the inner filamentous core, and only one row is included. This diagram is closely modeled after the mitochondrial cross-section shown in Fig. 1. Some of the prisms have been omitted from the foreground.

that invaginations of the inner mitochondrial membrane form foliate cristae oriented along the longitudinal axis of the organelle. Furthermore, when these foliate cristae approach the region of the mitochondrion which contains prisms and dot arrays, they assume angular configurations (Figs. 1, 6).

The results of the morphometric study reveal that about 10 mitochondrial profiles occur in the average muscle fibre at a given level of section and account for nearly 2% of the total muscle volume (Table I). About 40% of the mitochondria contain predominantly prismatic cristae. The average size of a prismatic mitochondrion does not differ significantly from that of normal mitochondria (Table I). There is, however, a significantly greater (75%) surface area of the cristal membranes in prismatic mitochondria compared to normal mitochondria (Table II).

DISCUSSION

The present observations establish that mitochondria with prismatic cristae occur in a representative of the Arthropoda, where they have hitherto not been reported. Our results are consistent with the

hypothesis suggested by Korman et al. (4) and expanded by Morales and Duncan (7) that an ordered packing of the matrix is involved in determining the angular cross sections of the cristae. We postulate that the matrices of the mitochondria are tightly packed with rodlets which course in parallel along the long axis of the organelle. The rodlets are composed of an electron-dense filament core (seen as the matrix dot array in cross-sections and as fine lines in longitudinal sections) and a less electron-dense shell. The idea of filaments with electron-dense cores and electron-lucent outer shells has been suggested (11) in connection with paracrystalline inclusion bodies in human liver mitochondria. The radius of these hypothetical rodlets is one-half the mean distance between adjacent dots measurable in cross section. Close packing of these rodlets results in the regular hexagonal dot array observed in cross sections. Using this basic structural model, we can account for (a) the triangular profiles, (b) the rhomboid profiles, (c) aberrant profiles such as extra-large triangles, (d) the array of triangle profiles, and (e) the two major types of rhombus arrays.

TABLE I

Composition of Vas Deferens Sphincter Fibers

Average area of muscle fiber (μm^2) 108.7 ± 17.8			
	Area fraction of mitochondria	Number of mitochondrial profiles per average area of muscle fiber	Average area of mitochondrial profile
	μm^2		μm^2
Total	1.9 ± 0.4	10.2 ± 2.0	0.17 ± 0.01
Prismatic type	0.9 ± 0.2	4.0 ± 1.0	0.23 ± 0.02
Normal type	1.0 ± 0.2	5.8 ± 1.1	0.18 ± 0.02

	Mitochondria	Nuclei	Vesicles	Myofibrils and cytoplasm
Volume density	1.8 ± 0.3	3.3 ± 1.8	1.4 ± 0.3	93.4 ± 15

Values given are means ± standard error of the mean; 30 fibers were examined.

TABLE II

Surface-to-volume Ratios of Average Prismatic and Normal Mitochondria*

	Normal‡	Prismatic‡	Dimension
Outer boundary membrane	10.6 ± 0.8	11.2 ± 0.8	m^2/cm^3
Cristal membrane	14.2 ± 2.1	24.7 ± 2.6§	m^2/cm^3

* Mean ± standard error of mean for 20 randomly selected mitochondrial profiles.
‡ Multiplied by a correction factor of 1.5 for membrane profile loss according to Loud (5).
§ $P < 0.001$.
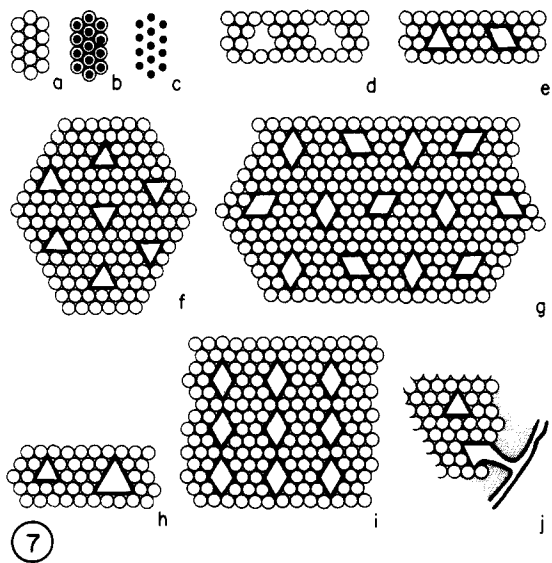
FIGURE 7   The rodlet deletion hypothesis. Close-packed fascicles of rodlets (a) with electron-dense cores and electron-lucent outer shells (b) result in the regular hexagonal dot array (c) seen in cross-section. Removal of a triangular fascicle of three or four rodlets results in holes in the array which have triangular or rhomboid outlines (d). When these holes are lined with cristal membranes, angular cristae result (e). The triangular-cristae array can be very regular (f), containing rows of cristae which intersect at angles of 60° or 120°. Two types of diamond-cristae arrays also result: one of these is the pinwheel arrangement (g), the other the diamond-back pattern (i). Invaginations of the inner mitochondrial membrane assume angular forms when they penetrate the rodlet array (j). Irregular forms such as extra-large triangles can also be explained by the model (h).

It can be seen (Fig. 7d) that deletion of a fascicle of three rodlets results in a hole in the rodlet matrix which has an equilateral-triangular cross-section. Similarly, deletion of a bundle of 4 rodlets leads to a rhomboid cross section. Lining of the perimeter of these spaces with cristal membranes (Fig. 7e) results in cristae with triangular or rhomboid profiles. This adequately accounts for the shape of the cristal profiles commonly encountered in the mitochondria of the sphincter muscle. It can also account for the aberrant profiles occasionally encountered in this and other preparations. For example, an extra-large prismatic cross-section is located near the center of a mitochondrion in Fig. 8 of Blinzinger et al. (2). The area of its cross-section is approximately double that of the surrounding cristae. Such a giant crista would result from the deletion of a triangu-

lar fascicle of six instead of three rodlets (Fig. 7h).

The relative spacing and orientation of cristal profiles with respect to one another is also explainable on the basis of a hexagonal rodlet model. Linear arrays of profiles intersecting each other at an angle of 60° or 120° would result, as can be seen in Fig. 7. The array of triangles and of rhombi which can be constructed resembles the patterns observed by us, with regard to both the angles between linear profile arrays and the relative orientation of the triangles and rhombi. The diamondback pattern of rhomboids (Fig. 5c) is also easily reproduced (Fig. 7i), as is the pinwheel arrangement (compare mitochondrion in lower left quadrant of Fig. 4 with Fig. 7g).

SUMMARY

Approximately 40% of the mitochondria in the sphincter muscle of the crayfish vas deferens have prismatic-type cristae. In cross section, the angular cristae have either triangular or rhomboid profiles which are surrounded by a hexagonal array of electron-dense dots. In longitudinal section, these mitochondria exhibit both thick and thin parallel lines, which represent cristae and filaments, respectively. It is postulated that the matrix of the prismatic-type mitochondria is packed with rodlets composed of an electron-dense core and a less dense shell. Close packing of these rodlets results in the regular hexagonal dot array. Deletion of fascicles of 3 or 4 rodlets results in spaces with triangular or rectangular cross sections. Lining of these spaces with membranes results in cristae with triangular or rhomboid cross sections.

REFERENCES

1. BLINZINGER, K., N. B. NEWCASTLE, and H. HAGER. 1964. Beobactungen über Mitochondrien mit eigenartiger Innenstruktur (Prisma-Typ) in Astrozyten des Goldhamstergehirns. Z. Naturforsch. Teil B. 19:514-515.
2. BLINZINGER, K., N. B. NEWCASTLE, and H. HAGER. 1965. Observations on prismatic-type mitochondria within astrocytes of the Syrian hamster brain. J. Cell Biol. 25:293-303.
3. FAIN-MAUREL, M. A. 1968. Variabilité de la struc-

ture mitochondriale dans les mucocytes des glandes salivaires de *Limnae stagnalis* L. (Gastéropode Pulmoné). *C.R. Hebd. Seances Acad. Sci.* **267:**1614–1616.

4. KORMAN, E. F., R. A. HARRIS, C. H. WILLIAMS, T. WAKABAYASHI, D. GREEN, and E. VALDIVIA. 1970. Paracrystalline array patterns seen in mitochondria. *Bioelectrochem. Bioenerget.* **1:**387–404.

5. LOUD, A. V. 1968. A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells. *J. Cell Biol.* **37:**27–34.

6. LUFT, G. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9:**409–414.

7. MORALES, R., and D. DUNCAN. 1971. Prismatic and other unusual arrays of mitochondrial cristae in astrocytes of cats and hamsters. *Anat. Rec.* **171:**545–558.

8. REVEL, J. P., D. W. FAWCETT, and C. PHILPOTT. 1963. Observations on mitochondrial structure. Angular configurations of the cristae. *J. Cell Biol.*
16:187–195.

9. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17:**208–212.

10. SUZUKI, T., and F. K. MOSTOFI. 1967. Intramitochondrial filamentous bodies in the thick limb of Henle of the rat kidney. *J. Cell Biol.* **33:**605–623.

11. THEMANN, H., and D. B. VON BASSEWITZ. 1969. Parakristalline Einschlusskörper der Mitochondrien des menschlichen Leberparenchyms. Elektronenmikroskopische und histochemische Untersuchungen. *Cytobiol.* **1:**135–151.

12. VAN HARREVELD, A. 1936. A physiological solution for fresh-water crustaceans. *Proc. Soc. Exp. Biol. Med.* **34:**428–432.

13. WEIBEL, E. R. 1969. Stereological principles for morphometry in electron microscopic cytology. *Int. Rev. Cytol.* **26:**235–302.

14. YOUSON, J. H. 1971. Prismatic cristae and matrix granules in mitochondria of the kidneys of ammocoetes. *J. Cell Biol.* **48:**189–191.