MICROCYLINDERS WITHIN MITOCHONDRIAL CRISTAE IN THE RAT PINEALOCYTE

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ABSTRACT

A minute cylindrical structure with a dense core, designated as "microcylinder," has been observed within enlarged spaces of mitochondrial cristae in pinealocytes of some adult rats (osmium tetroxide fixed, methacrylate embedded). The microcylinders are 270 to 330 A in diameter and of indeterminate length. Their wall is found to be made up of slender filamentous subunits, probably 6 in number, and surrounds a central filament. The microcylinders are arranged parallel to one another, forming monolayered or, more frequently, multilayered aggregates. Their number within a crista varies considerably. Packets of microcylinders may be seen located in the outer mitochondrial chamber, but are never found in the mitochondrial matrix. They have been observed neither in other cell types of the pineal gland nor in neurons, ependymal, and glial cells of the nearby epithalamic tissue. The origin and nature of the microcylinders are unknown. Glycogen-like particles have been encountered, though very infrequently, accompanying bundles of microcylinders within mitochondrial cristae.

INTRODUCTION

During a study of rat pineal glands, a unique cylindrical structure with a dense core has been noticed within enlarged spaces of mitochondrial cristae in pinealocytes of some animals. The term microcylinder is proposed to designate this type of inclusion. Another interesting finding is the presence of glycogen-like particles accompanying microcylinders within cristae. The occurrence of microcylinders within mitochondria has not previously been reported either in pinealocytes or in other cell types. The intramitochondrial bodies usually encountered are located in the matrix of the organelle and appear as electron-opaque particles or granules. Inclusions within mitochondrial cristae thus far reported are dense bodies in the hyperplastic mouse epidermis (5) and in the estrous uterine epithelium of mice (8, 13), hexagonal crystalline bodies in the oocyte of *Rana pipiens* (17), and helical filaments in astrocytes of rat corpus striatum (12). The microcylinder here reported is quite different from all those inclusions. Although the origin and nature of this peculiar structure are unknown for the time being, its occurrence and morphology deserve to be reported.

MATERIAL AND METHOD

Intramitochondrial microcylinders were found in the pineal glands of two out of 18 rats of Long-Evans strain sacrificed while under various experimental or physiological conditions. The two rats, one female weighing 243 gm and one male 350 gm, came from a different batch than that of the rest of the animals studied, showed no remarkable findings on inspection at autopsy, and had not received any particular treatment prior to sacrifice. The pineal glands and small pieces of epithalamic nervous tissue, including the subcommissural organ, were fixed in cold veronal-

acetate-buffered osmium tetroxide containing sucrose (1), dehydrated in graded alcohols, and embedded in butyl and methyl methacrylate (6:1). Sections with silver or yellow interference colors were mounted on carbon-coated grids and stained with lead after the method of Dalton and Zeigel (3) or Karnovsky (10). Electron micrographs were made at initial magnifications of 6,000 to 37,000 diameters with a Hitachi HU 11 electron microscope.

OBSERVATIONS

All the figures illustrate the cytoplasmic bodies containing microcylinders found in the perikaryon of pinealocytes. Variations in their appearance depend upon their size and planes of section. The bodies consist of a double limiting membrane and internal single membranes enveloping packets of minute cylinders. These structures suggest that the bodies are unusual mitochondria within the enlarged cristae of which microcylinders have been developed. In some instances, the bodies have a region which shows ordinary, not dilated mitochondrial cristae. The membrane of such cristae is seen, on occasion, to be continuous with the internal mitochondrial membrane (Fig. 1). The finding supports the assumption that the microcylinder-containing bodies are derived from mitochondria. The bodies are usually elongate rods or spindles, and vary considerably in size.

Transverse, longitudinal, and oblique sections of the inclusion reveal that it is cylindrical, 270 to 330 A in diameter and of indeterminate length, constructed of a dense wall and a dense core. At high magnification, the wall of the microcylinders is found made up of slender filamentous subunits, about 80 A in diameter and probably 6 in number, surrounding an axial one. In cross-section, the

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All figures are from sections of the pinealocyte of an adult female rat which were stained with lead by the method of Karnovsky (10) except for the section shown in Fig. 4 which was contrasted after the method of Dalton and Zeigel (3).

FIGURE 1 A microcylinder-containing body, apparently showing characteristic features of mitochondria. Note a double limiting membrane and several cristae, one of which is dilated and contains an aggregate of microcylinders in transection view. × 67,000.

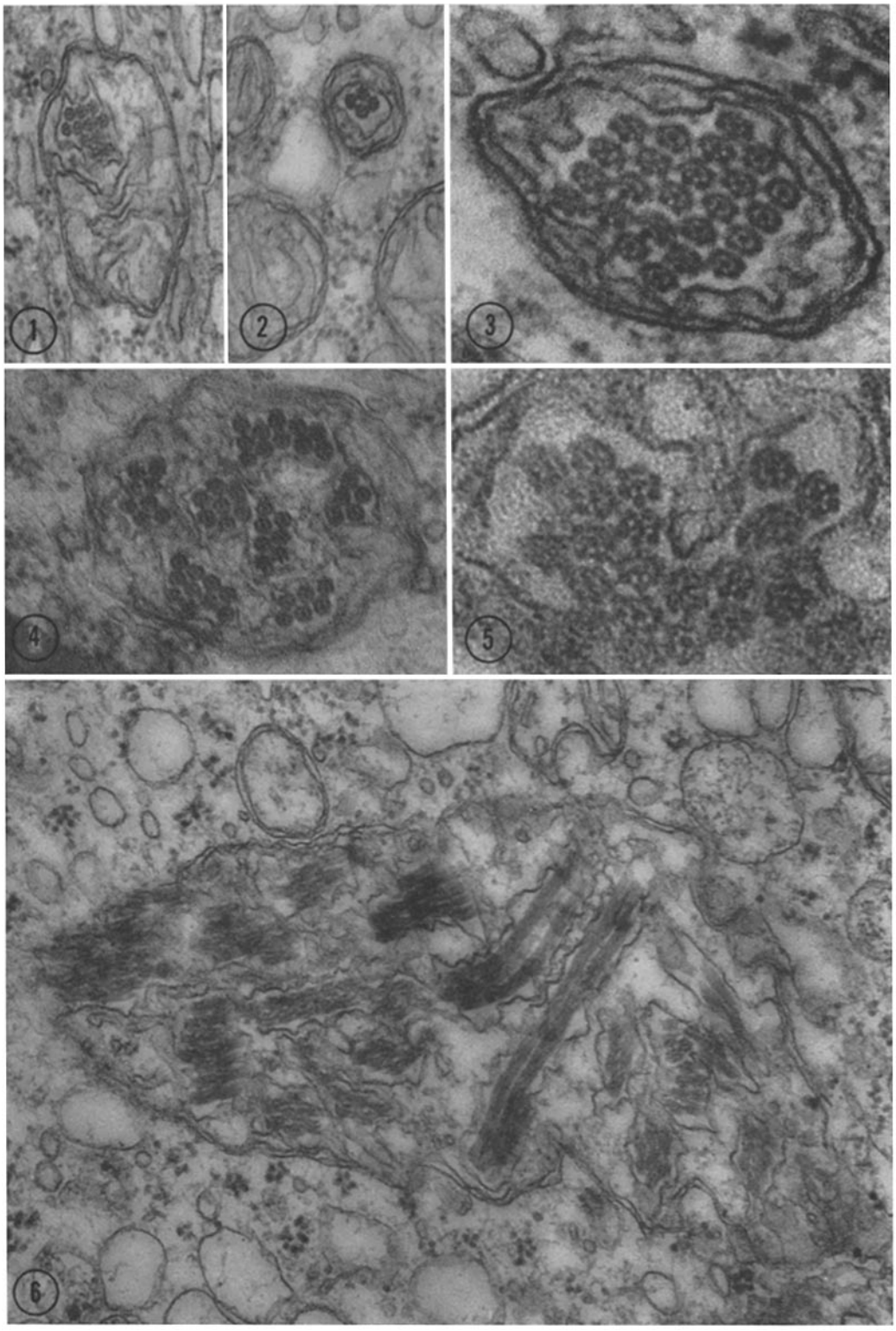
FIGURE 2 Portions of three unchanged mitochondria and a microcylinder-containing one. The latter is the smallest one of this type encountered in this study. Four round profiles of microcylinders are seen enveloped by a loosely fitting, dense membrane interpreted as the membrane of a crista. × 65,000.

FIGURE 3 A cytoplasmic body bounded by two dense membranes, believed to be derived from a mitochondrion. In the interior a multilayered aggregate of 24 microcylinders, cut nearly transversely, is seen surrounded by a ruffled membrane homologous to a mitochondrial crista. The aggregate apparently shows a regular hexagonal array of microcylinders, each of which appears to be made up of a dense wall and a dense core. Instead of being uniform in thickness, the wall of most microcylinders appears composed of several knobs interpreted as transverse sections of filamentous substructures. × 167,000.

FIGURE 4 Transverse section of a microcylinder-containing mitochondrion. Five irregularly enlarged spaces of cristae are discernible. Multilayered aggregates of microcylinders are obvious, and hexagonal packings are visible in some places. × 75,000.

FIGURE 5 High magnification of an aggregate of microcylinders in transverse section. Some of the profiles show that the microcylinder is made up of probably 6 peripheral subunits and a single central one. Tiny bridges are discernible between them. Each subunit shows a dense wall and a less dense interior suggesting the tubular nature of the filament. × 197,000.

FIGURE 6 A large microcylinder-containing mitochondrion. In the interior of this body there are many ruffled membranes enclosing packets of microcylinders in variable numbers which are sectioned in various planes to their long axis (see text). × 62,000.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central filament and peripheral ones appear to be connected by minute bridges (Fig. 5). Because of the limitations of the present material and method, their finer structure and exact relations have yet to be worked out. Figs. 7 and 8 depict longitudinal sections of small packets of microcylinders. The fibrillar appearance can be attributed to the longitudinal view of the filamentous subunits.

In available micrographs, microcylinders in packets vary from 4 to 30 in number, and are arranged parallel to one another, forming monolayered or, more frequently, multilayered aggregates. When in large enough numbers, they appear in close hexagonal packing in transection view (Figs. 1, 3, 4). They seem to vary considerably in length, and, in many instances, appear to extend uninterruptedly over the whole length of the mitochondrion. Irrespective of the length, their long axis is usually parallel to that of the mitochondrion. Therefore, the mitochondrial cristae containing packets of microcylinders are usually dilated much more extensively in the direction parallel to the long axis of the mitochondrion than in the transverse direction. The cristae also vary in number and size within a single mitochondrion (Figs. 1 to 4, 6). The cristal membrane is not tightly fitting on the bundle of microcylinders, but usually appears as a ruffled membrane (Figs. 1, 3, 6, 7).

In the majority of instances, the packets of microcylinders are arranged parallel to one another and to the long axis of the mitochondrion. Occasional mitochondria display a somewhat random orientation of the bundles of microcylinders, as shown in Fig. 6. This appearance, however, may result from cutting through a branching or bending mitochondrion, since such profiles are encountered elsewhere in pinealocytes. With an increasing number of the microcylinder-containing cristae, the whole mitochondrial body usually appears to increase in diameter and in length. However, mitochondria with a small diameter, containing a few microcylinders, may have a considerable length (Fig. 8). In available micrographs, the diameter measures up to 1.3 μ and the length up to 5.8 μ. No substantial changes appear in the mitochondrial membranes as the whole body gains in size. Regardless of the number of the cristae, the matrix may occasionally become large in amount and contain a fine fibrillar substance of a moderate density.

In a few instances, a fascicle of microcylinders is seen dislocated in a dilated outer mitochondrial chamber. Another rare appearance is a bundle of microcylinders surrounded by a single limiting membrane. It looks like a free or isolated microcylinder-containing crista in the cytoplasm instead of being located within a mitochondrion. This profile may be attributed to a section cut through a dilated outer mitochondrial chamber containing the above-mentioned dislocated microcylinders, which leaves the remaining portion of the mitochondrion outside the thickness of the section.

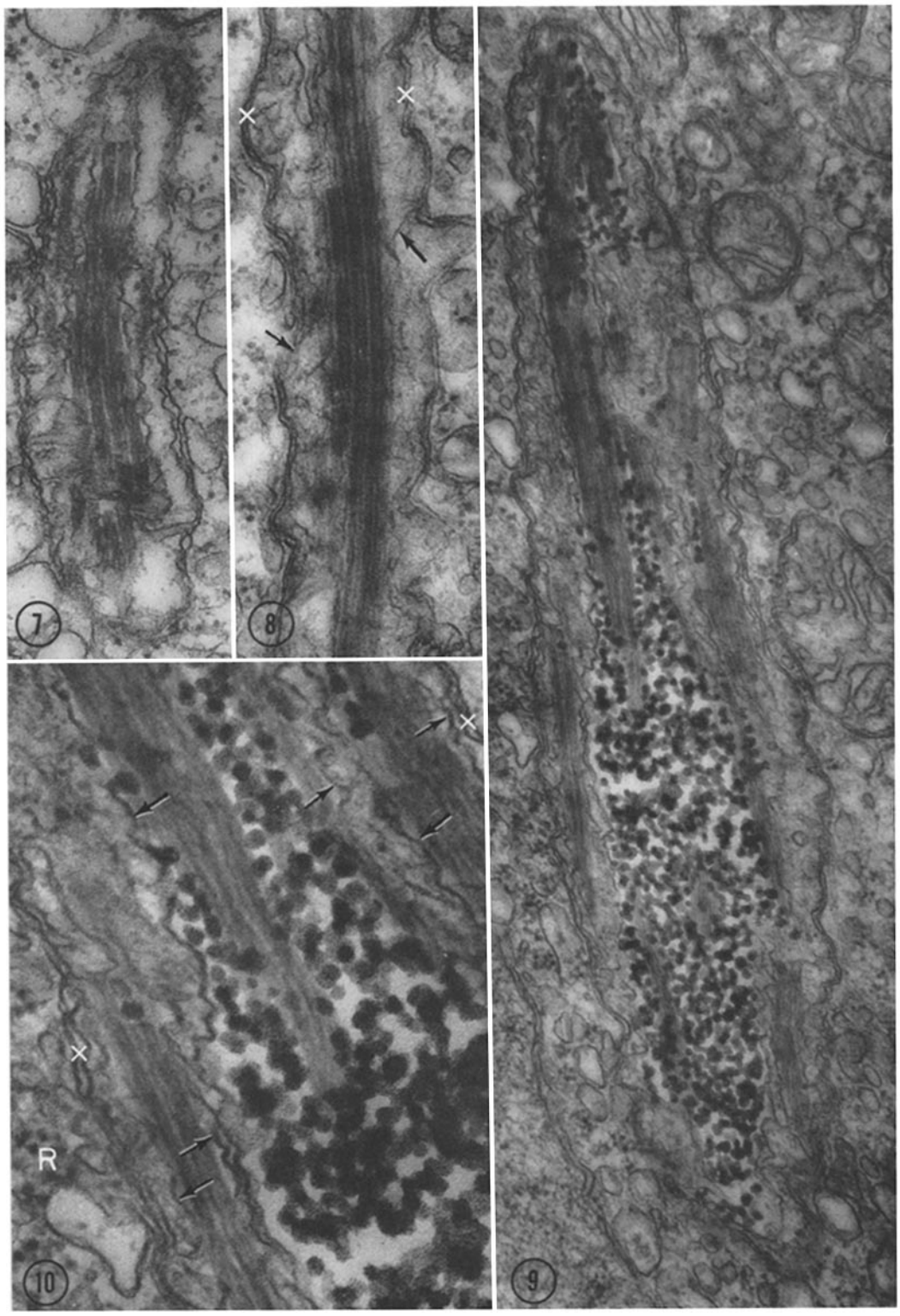
Fig. 9 shows an interesting variant of micro-

FIGURE 7 Longitudinal section of a microcylinder-containing mitochondrion. Note a dense limiting membrane and a ruffled cristal membrane enveloping elongated profiles of microcylinders. Fibrillar appearance of cylinders is discernible. × 60,000.

FIGURE 8 A portion of an elongated mitochondrion which measures 2.8 μ in length. Three longitudinally sectioned microcylinders show filamentous subunits. Arrows, cristal membrane. *x*, external and internal mitochondrial membranes. × 72,000.

FIGURE 9 A large mitochondrion containing unusual inclusions. In its enlarged intracristal spaces there are bundles of microcylinders in nearly longitudinal section and numerous dense particles. The dense particles are unevenly distributed between the bundles of microcylinders. Several normal mitochondria are also shown in the upper right quadrant of this figure, and a small portion of the nucleus in the lower left corner. A portion of this figure is enlarged in Fig. 10. × 52,000.

FIGURE 10 High magnification of a portion of Fig. 9. In addition to microcylinders, numerous particles are seen aggregated at random. Close inspection of the particles reveals their stippled appearance, which is essentially identical to that of glycogen particles previously identified (*cf.* Fig. 6 in 16). Arrows, membranes of cristae. *x*, external and internal mitochondrial membranes. *R*, ribosomes in the cytoplasm. × 111,000.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cylinder-containing mitochondria. In addition to microcylinders, there are numerous dense particles about 300 A in diameter within the cristae. They are not observed in the mitochondrial matrix. Higher magnification reveals that the dense particles are made of fine dense granules embedded in a less dense substance (Fig. 10). Since the particles have a diameter of about 300 A and stain intensely with lead and their stippled appearance is essentially identical to that of glycogen particles previously depicted by other workers (15, 16), they are tentatively identified as glycogen. Only two mitochondria containing such particles have been encountered so far in the present material. Although traces of glycogen have been detected by light microscopy in the cytoplasm of pinealocytes of the rhesus monkey (19), no electron microscopic evidence for the presence of glycogen has been shown in the cytoplasm of rat pineal cells in the present material or in the material of previous workers (4, 6, 7).

Although in a given pinealocyte the microcylinders are absent from most mitochondria, it is not very difficult to find them in the organelles of the perikaryon because their aggregates possess considerable electron opacity. Free microcylinders have not been observed either in the cytoplasmic matrix or elsewhere. Microcylinders occur neither in the mitochondria of the cytoplasmic processes of the pinealocyte nor in those of other pineal gland cells. No microcylinders were found in the mitochondria of neurons, glial and ependymal cells in sections of the subcommissural organ and adjacent brain tissue taken from the same rats.

DISCUSSION

Although the occurrence of giant and pleomorphic mitochondria in the rat pinealocyte has been noted (6, 7), such intracristal cylinders as those described in this paper have not been reported. Microcylinders have been found in only two out of 18 rats examined by the author to date. They occur only in the mitochondria of the perikarya of pinealocytes, and are absent from those in either other cell types of the glands or cells in the nearby epithalamic nervous tissue, including ependymal cells and subcommissural organ cells. As no preparations were made on other portions of the brain or other organs from these two rats, it is impossible to decide whether or not the occurrence of microcylinders is confined to the pineal gland.

The significance of intramitochondrial bodies has been variously speculated on, for example: the dense granules in the mitochondrial matrix of the duodenal absorptive cell were assumed to represent aggregates of cations (18); the *corpus intra cristam* in the cells of the hyperplastic epidermis was found to act as a center of agglomeration of tonofilaments (5); the intramitochondrial bodies in estrous uterine epithelium was related to an accelerated cell metabolism (8); the hexagonal crystalline bodies in the cristae were assumed to play a direct role in the synthesis of yolk in the oocyte of the frog (17); the helical filaments in cristae found in the rat astrocyte were considered to represent a fibrous protein synthesized within mitochondria (12); the presence of trabecular ("*balkenförmig*") structures in the mitochondria of the bat hepatic cell was related to the functional demand of the mitochondria during hibernation (2). The origin and nature of the microcylinders reported in this paper remain unknown for the time being. As the microcylinders are apparently of rare occurrence in the pineal gland, as stated above, it seems likely that they are not intrinsic components of the rat pinealocyte, and they may represent proteinaceous products of an unusual mitochondrial activity. The high concentration of biogenic amines in the pineal gland is correlated with the presence of "plurivesicular" component of the pinealocyte (4); hence the localization of the amines in mitochondria seems unwarranted. The rare occurrence of the microcylinders also excludes the possibility that the amines are directly associated with them.

According to Pease (14), the walls of the flagellar fibrils or cylinders of rat sperm are made up of 10 longitudinally oriented filaments, and Ledbetter and Porter (11) reported that in the microtubules in the cortices of certain plant cells is found a wall made up of slender filamentous subunits, probably 13 in number. The latter authors suggest that these types of cytoplasmic tubules may be homologous and possess similar functions, primarily of motion. It is of interest to note that the walls of the microcylinders, the diameter of which is comparable to that of the microtubules, are also constructed of longitudinally oriented filamentous subunits. The flagellar fibrils and the microtubules, however, differ from the microcylinders in the number of the filamentous subunits, and they are also distinct from the microcylinders in that they have a less dense core instead of a central filament. It seems unlikely

that the microcylinders are functionally analogous with both the flagellar fibrils and the microtubules.

Glycogen particles usually appear in the cytoplasmic matrix, and their occurrence within mitochondrial cristae is obviously very uncommon. As the presence of glycogen particles unaccompanied by microcylinders within mitochondrial cristae has been reported also in the retinal receptor cells of the rat (9), it seems likely that the occurrence of glycogen within the cristae does not necessarily depend upon the presence of microcylinders. The presence of glycogen particles within mitochondria suggests that the enzymes related to glycogenesis do exist at least in the particular mitochondria, although a survey of the biochemical literature reveals that no paper describing the presence of such enzymes in mitochondria has appeared to date. In the absence of knowledge of the nature of microcylinders, it is premature to place an interpretation on the intimate association of the mitochondria, microcylinders, and glycogen particles.

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