HELICAL FILAMENTS IN ASTROCYTIC MITOCHONDRIA OF THE CORPUS STRIATUM IN THE RAT

ENRICO MUGNAINI, M.D.

From the Anatomical Institute, University of Oslo, Oslo, Norway. Dr. Mugnaini's present address is the Anatomical Institute, University of Bergen, Bergen, Norway

ABSTRACT

Astrocytic mitochondria have been studied in serial electron micrographs of the corpus striatum. Special filaments have been found inside several of these mitochondria located in both the cell body and the processes. They were particularly frequently observed in the pericapillary end-feet. The filaments occur within dilated intracristal spaces provided with several communications with the outer chamber, and form helices which are oriented approximately in the same direction. Each filament is about 30 A thick, the diameter of the helix is 140 A, and the pitch is 120 A. Their possible nature and significance are briefly discussed. There is no clear relationship between these mitochondria and other unusual forms of mitochondria previously described in astrocytes from other regions.

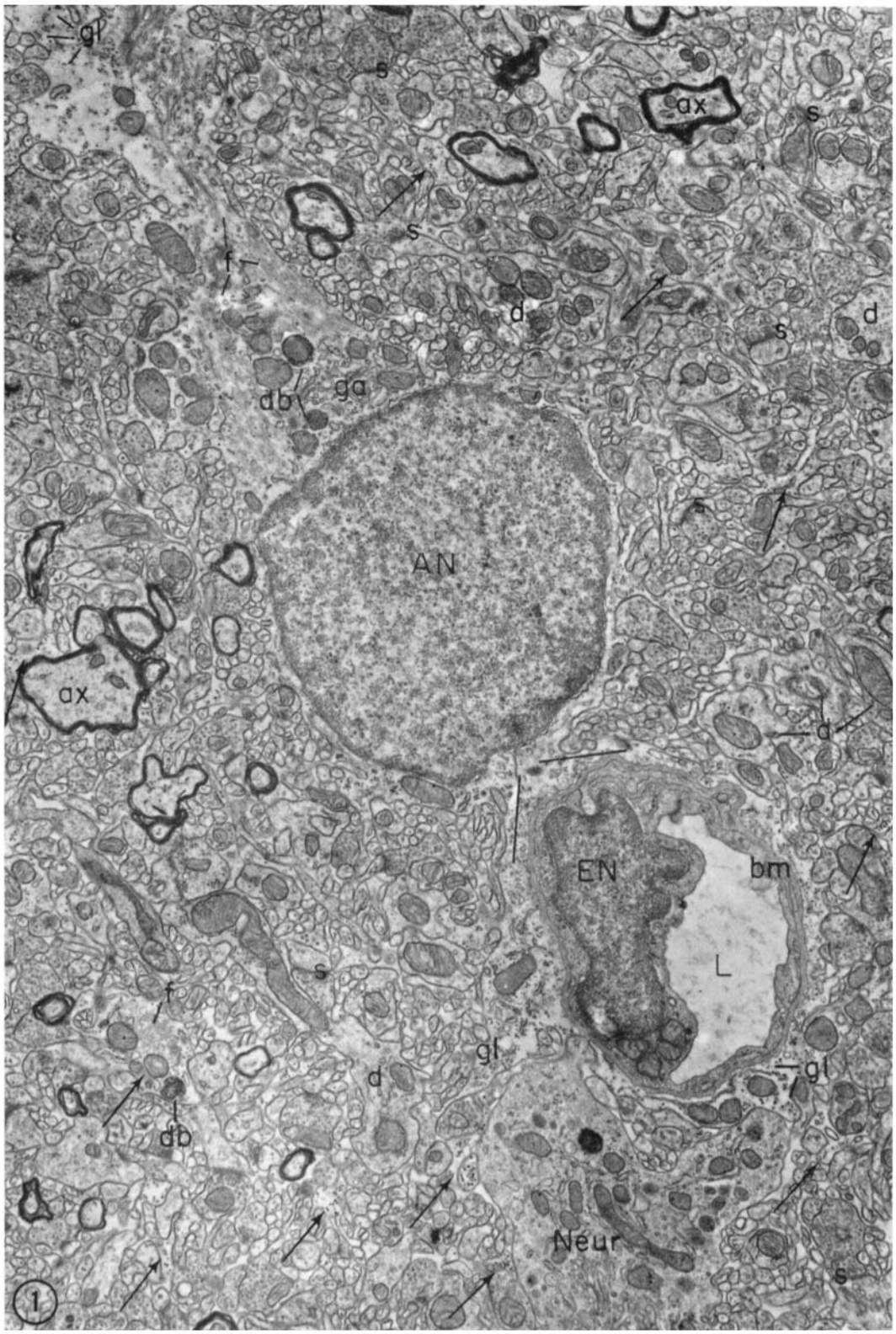
INTRODUCTION

In the course of electron microscopic studies on the central nervous system, unusual forms of mitochondria have been found. Mitochondria with few cristae and with fibrous elements in the matrix have been seen by Hartmann (11) and Farquhar and Hartmann (6) in marginal glial cells, by Gray (9) in neuroglial cells said to contain fibrils, and by Mugnaini and Walberg (15) in cells defined as astrocytes. All of these findings were obtained from the cerebral cortex of the adult rat. Furthermore, mitochondria with a highly regular internal structure were described by Gray (10) in glial cells containing fibrils, in the lizard brain, and by Mugnaini and Walberg (15) in fibrous astrocytes from the inferior olive of adult cats.

This paper deals with mitochondria of a peculiar structure which have not been described previously. They have been found in glial cells classified as astrocytes. Therefore a brief description of the more usual form of mitochondria in these cells is also given. Astrocytes can be readily recognized because of several characteristics (see also references 3, 20, 15). They have many processes, some of which form perivascular end-feet. The cytoplasm is clear, although, in good preparations, not "watery," and contains many glycogen-like particles. Typical fibrils are often seen as well as various kinds of dense bodies.

MATERIALS AND METHODS

A number of albino rats, of both sexes, weighing 300 to 430 gm were used. The brain was taken out under Nembutal anesthesia. The hemispheres were rapidly separated from each other and then cut into two parts with a razor blade. This last cut was made in a frontal, sagittal, or horizontal plane through the corpus striatum. Afterwards the pieces were immersed in chilled 2 per cent osmium tetroxide fixative. The latter was buffered with Veronal-acetate (19), or with phosphate according to Millonig (14), and 4.5 per cent sucrose (4) or 0.54 per cent glucose (14), respectively, was added. Small, well fixed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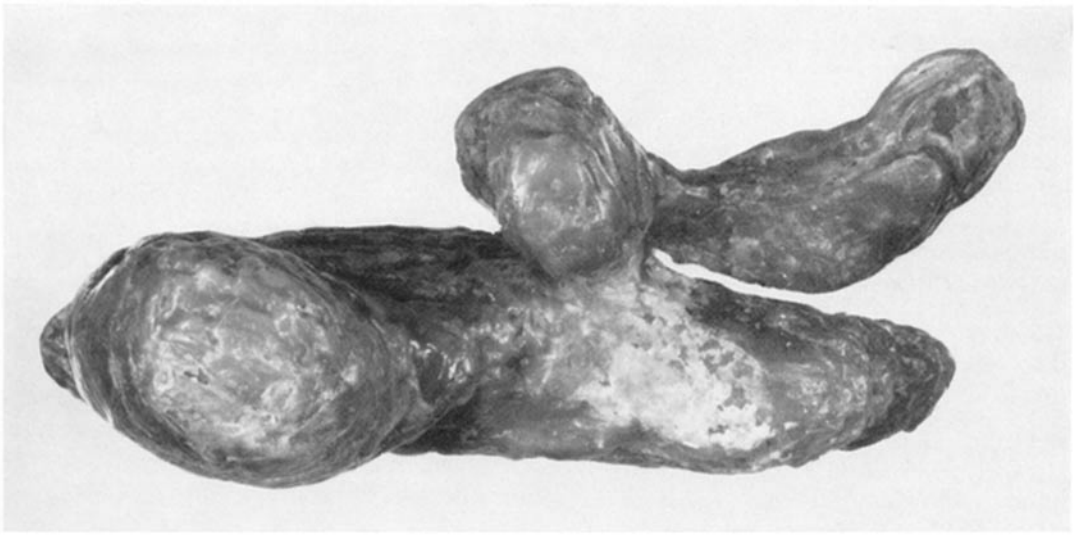


FIGURE 2 Model of an irregularly shaped mitochondrion built of dental wax. The projection was connected with the remainder of the body through a narrow stalk only (reproductive activity?). The mitochondrion is the same as the one labeled *m* in Figs. 3 to 5. For the reconstruction, 32 ultrathin sections were used. Each section has been regarded as 500 A thick.

Abbreviations to Figures

AEF, astrocytic end-foot
AN, astrocytic nucleus
ax, myelinated axon
bm, capillary basement membrane
d, dendrite
db, dense body
EN, endothelial cell nucleus
end, endothelial cell cytoplasm
er, endoplasmic reticulum
f, glial fibrils
ga, Golgi apparatus
gl, glycogen-like granules
L, lumen of blood capillary
m, mitochondrion containing helical filaments
Neur, neuronal perikaryon
p, mitochondrial projection
s, synapse
For significance of asterisks, arrows, and other symbols, see legend to the individual figures.
Figs. 3, 4, 5, and 6 represent sections 13, 20, 25, and 49 of a series of 67 ultrathin sections. Figs. 1, 7, and 8 belong to three other series.

FIGURE 1 Electron micrograph showing a protoplasmato-fibrous astrocyte embracing, with two processes (at unlabeled straight lines), a blood capillary. Mitochondria, Golgi apparatus (*ga*), smooth- and rough-surfaced endoplasmic reticulum, dense bodies (*db*), glial fibrils (*f*), and scattered glycogen-like granules (*gl*) are present in the cytoplasm. At *AN*, astrocytic nucleus. In the neurophil, several synapses (at *s* and elsewhere), dendrites (*d*), and astrocytic processes (arrows) are seen (labeled only in part). Numerous boutons, with synaptic vesicles, are unlabeled. The astrocytic processes are readily identifiable because of the presence of glycogen-like granules (200 to 400 A wide) well shown by the lead staining. Even at this low magnification, a mitochondrion with a clear space containing some structures is seen in an astrocytic process (lower left). Portion of a neuronal perikaryon (*Neur*) is included in the micrograph. At *ax*, myelinated axon; *L*, lumen of the capillary; *bm*, basement membrane; *EN*, endothelial cell nucleus. × 11,000.

pieces from different parts of the corpus striatum (taken from the cut surface) were selected under a dissecting microscope during dehydration in cold acetone and embedded in Araldite. Serial ultrathin sections were made with an LKB ultramicrotome and mounted on copper diaphragms provided with an 800 μ hole covered with a Formvar film reinforced with carbon. The sections were stained with lead (12) and micrographed with a Siemens Elmiskop 1b. In addition, stereoscopic micrographs were examined. Models of mitochondria were built in dental wax, as done by Westrum and Blackstad (27).

OBSERVATIONS

The astrocytes of the corpus striatum from adult rats are the protoplasmic and the protoplasmato-fibrous types (see also Mugnaini and Walberg, reference 15). Since there is a large spectrum of intermediate stages between protoplasmic and fibrous astrocytes, the classification of a single cell as protoplasmic or protoplasmato-fibrous can not always be made. There are fibrous astrocytes intermingled with compact bundles of fibers of the internal capsule, but these will not be considered in this paper.

Mitochondria are generally numerous in astrocytes of the corpus striatum and are encountered in both the cell body and the processes (Fig. 1). They are usually round or moderately elongated; however, very long mitochondria, sometimes exceeding 9 μ in length, are found. These are longitudinally oriented in the processes but have a variable orientation in the cell body and the end-feet. Other mitochondria are irregularly shaped, sometimes having a projection that is connected with the parent organelle by a narrow stalk. Fig. 2 is a wax model of such a mitochondrion, *viz.*, the one labeled *m* in Figs. 3 to 5. The stalk may be so thin that it is seen only in two or three subsequent serial micrographs. Thus the external morphology of the mitochondria in these astrocytes is not significantly different from that of mitochondria in other cells in nervous tissue. Also, the matrix is of about the same density as that of mitochondria in other neural structures, and seems to be the same in the cell body as in the processes (Fig. 1). In the matrix one or more dense granules may be encountered which are similar to those seen in mitochondria from other tissues (Figs. 1, 3 to 8).

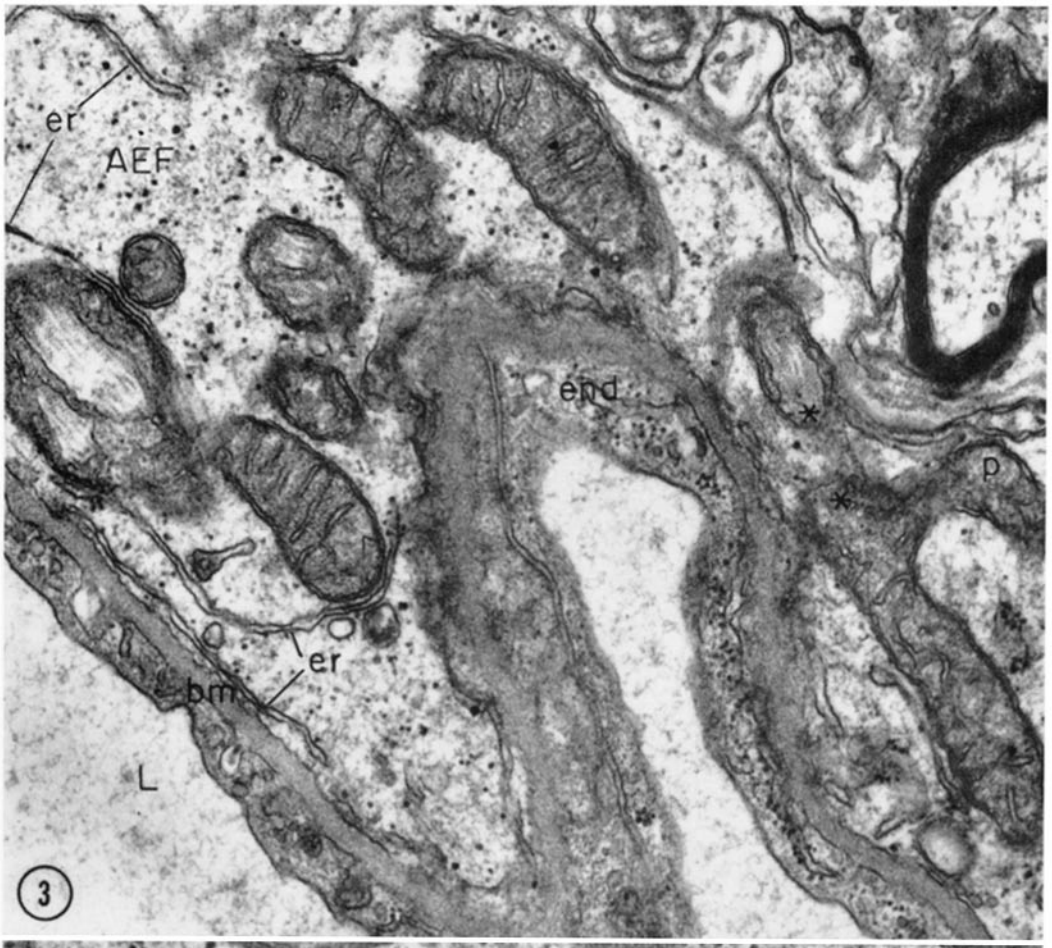
The arrangement and length of the cristae are variable and not specific. A number of the longer cristae either branch or reach the opposite side of the mitochondrion, dividing the inner chamber into compartments. Therefore, all four types of cristal arrangement listed by Grassé *et al.* (8) may be found. Sometimes the cristae show an angular configuration as described by Revel and Fawcett (24). In some mitochondria the concentration of the cristae is moderately high. In others only few cristae are present, leaving the inner chamber of the organelle occupied chiefly by granular matrix. None of the unusual forms of mitochondria described in other regions were seen in the astrocytes of the corpus striatum.

Although the mitochondria generally showed the usual ultrastructural appearance just described, in many instances very special features were observed. In such mitochondria the intracristal spaces are widely dilated forming clear compartments containing helical structures. At many places these compartments communicate with the outer chamber (Figs. 5, 6, and 8). Sometimes 2 to 4 such communications are seen in a single micrograph. In other micrographs the dilated spaces with the helical structures are not connected with the outer chamber (Fig. 7), or such connection is seen only at one place (Fig. 6). Examination of serial sections, however, reveals additional communications at other levels of the series.

The mitochondria displaying these special features tend to be larger and to have more bulbous

FIGURE 3 Part of a blood capillary surrounded by an astrocytic end-foot. Helical structures are clearly distinguishable in the intracristal spaces of some mitochondria. At *p*, a projection arising from a mitochondrion. The examination of serial micrographs reveals that the profiles labeled with the asterisks are part of the same mitochondrion and that the structures labeled *er* are elongated cisternae of endoplasmic reticulum. The mitochondrion labeled *m* is the same as the one shown in Figs. 4 and 5 at different levels. × 45,000.

FIGURE 4 Mitochondria with helical structures (shown at a different level in Fig. 3). At *p*, the neck of a projection. × 60,000.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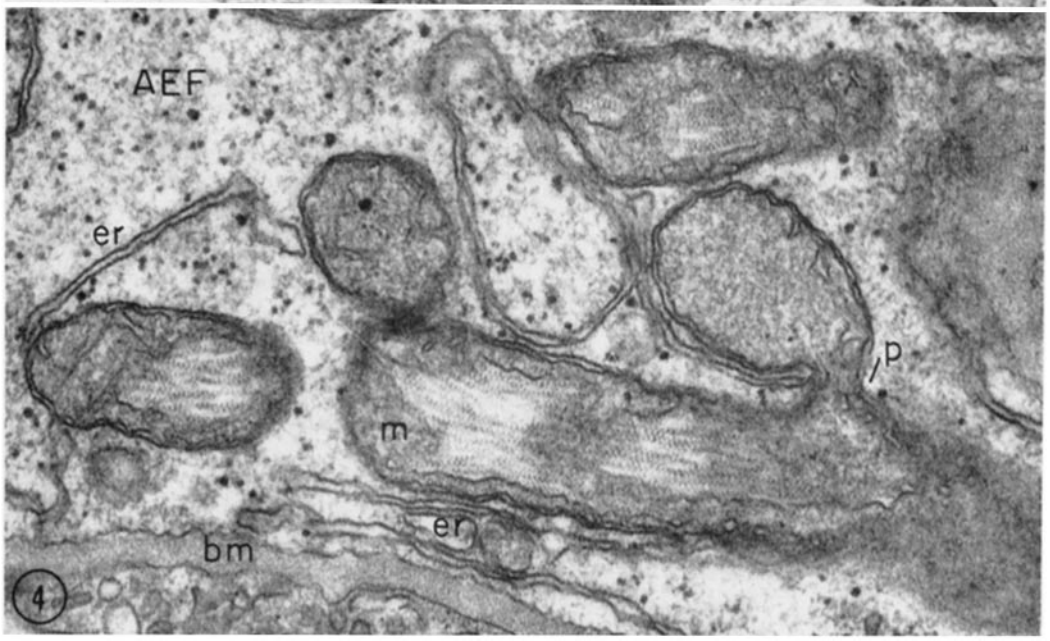
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projections than usual for mitochondria within these cells.

The number of helices present in a single mitochondrion varies greatly. Sometimes, one or more dilated intracristal spaces with many helices almost completely fill up the mitochondrion (Figs. 3 to 5 and 8). No definite discontinuity has been observed either in the membrane limiting the dilated spaces or in the external limiting membrane of the mitochondria. Apparent discontinuities may easily be explained as tangential sectioning of the membranes.

Longitudinal (Fig. 5) oblique and cross-sections (Fig. 7) of the mitochondria examined at high magnification show that the helical structures are filaments, separated from each other and oriented approximately in the same direction. The stereoscopic micrographs reveal that at least a majority of the helices have a right-hand screw axis. Each filament is about 30 A thick, the diameter of the helix is about 140 A, and the pitch is 120 A (Fig. 9). The length of a helix included in one micrograph has often been seen to be as much as 0.6 μ (Fig. 8).

Helix-containing mitochondria were encountered in the cell body (Fig. 8) and in the processes lying in the neuropil, and particularly frequently in the pericapillary end-feet (Figs. 3 to 6 and 8). Some of them were in close apposition to an elongated cisterna of endoplasmic reticulum (Figs. 3, 4, and 7). Helix-containing mitochondria have been observed in all the animals studied, in both sexes. Various kinds of lysosome-like dense bodies (Fig. 1) are present in astrocytes (for a survey, see Mugnaini and Walberg, 15), but no intermediate forms between these and the filament-containing mitochondria have ever been seen.

DISCUSSION

To the author's knowledge, mitochondria with helical filaments, as observed in this study, have never been described before. It could be debated whether these filaments exist as such in the living state or arise during the preparation of the tissue for electron microscopy. It is known that coarse artifacts may be produced during this process, and the configuration, for instance, of protein macromolecules, may be influenced by such factors as pH, bound ions, the solvent, and the concentration of the protein itself (13). However, the filaments appeared identical in all preparations, independent of the buffer solutions used, and it seems reasonable to accept them as real.

Their role in the mitochondrion is an intriguing problem. Inclusions of various material within mitochondria have been shown to occur in different types of cells (see Novikoff, reference 18, for bibliography). In Testacella, André (2) even observed filaments in the intracristal space of spermatocyte mitochondria. However, none of the intramitochondrial structures seen so far resemble the helical structures here described and allow us to make any inference on their nature.

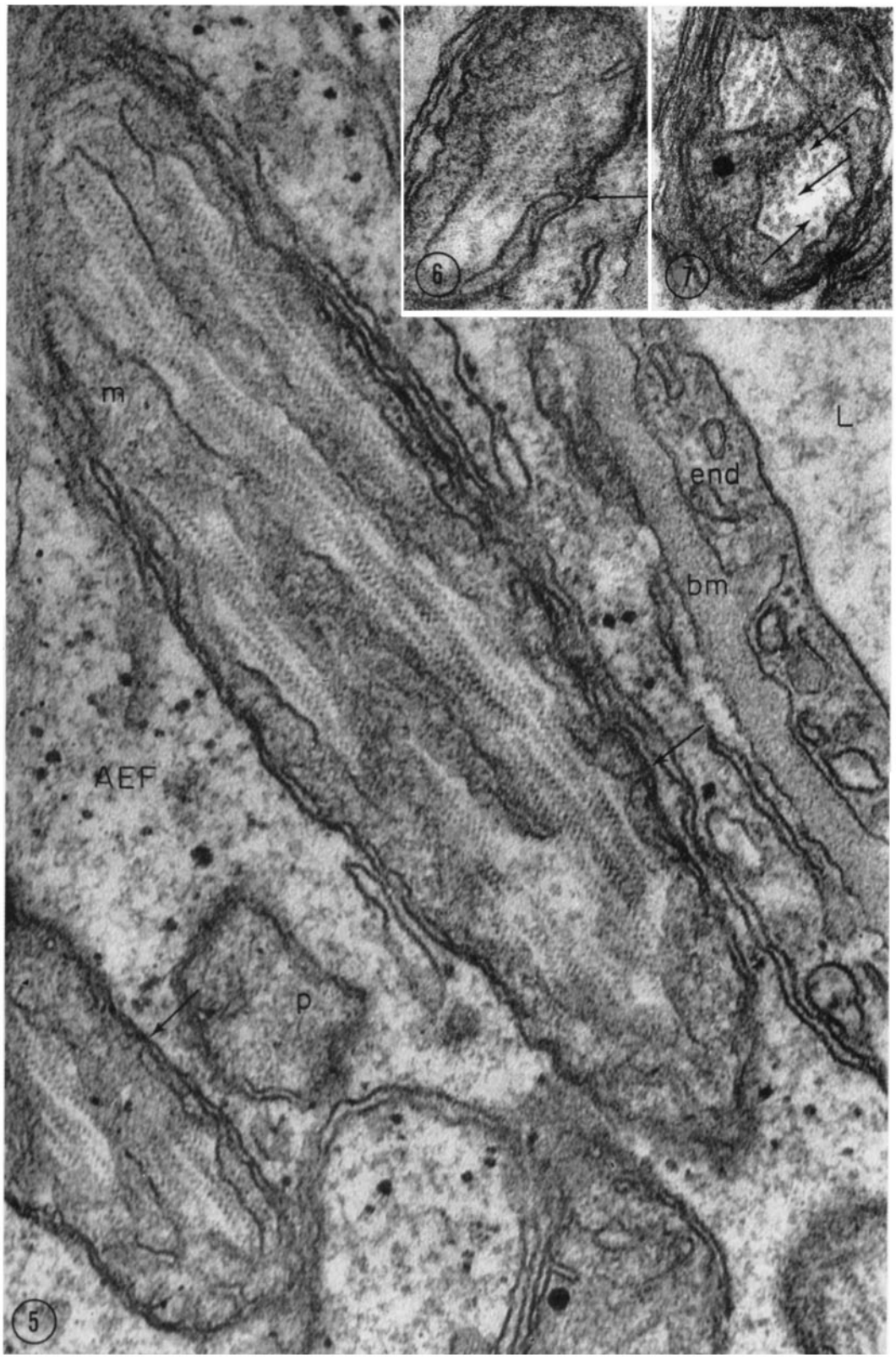
One could raise the question whether the mitochondria with the helical filaments are undergoing some kind of degeneration. This does not seem likely, since these mitochondria are of frequent occurrence and appear in cells displaying no other apparent sign of degeneration.

Thus, it seems a reasonable hypothesis that the helical filaments are macromolecules of a material, probably of protein nature, synthesized under normal conditions within the astrocytic mitochondria. It has indeed been shown earlier that in some cells protein synthesis is one of the

FIGURE 5 Same mitochondrion as in Figs. 3 and 4 but at a different level. The helical structures contained in the intracristal spaces at this higher magnification appear to be single filaments about 30 A thick. Arrows indicate communications between intracristal spaces and the outer chamber. At *p*, the projection connected with the parent body at the level of the preceding micrograph. × 100,000.

FIGURE 6 Communication between a dilated intracristal space and the outer chamber (arrow). In subsequent micrographs other communications appear. × 100,000.

FIGURE 7 Mitochondrion (from an astrocytic end-foot) in which one of the two dilated intracristal spaces contains cross-sectioned filaments, separated from each other (arrows). × 100,000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many ATP-utilizing processes that can occur in mitochondria (18).

The predominance of the helix-containing mitochondria in pericapillary end-feet is a striking phenomenon which may well be of functional importance. The same may be said about their close proximity to endoplasmic reticulum, as was observed in some instances.

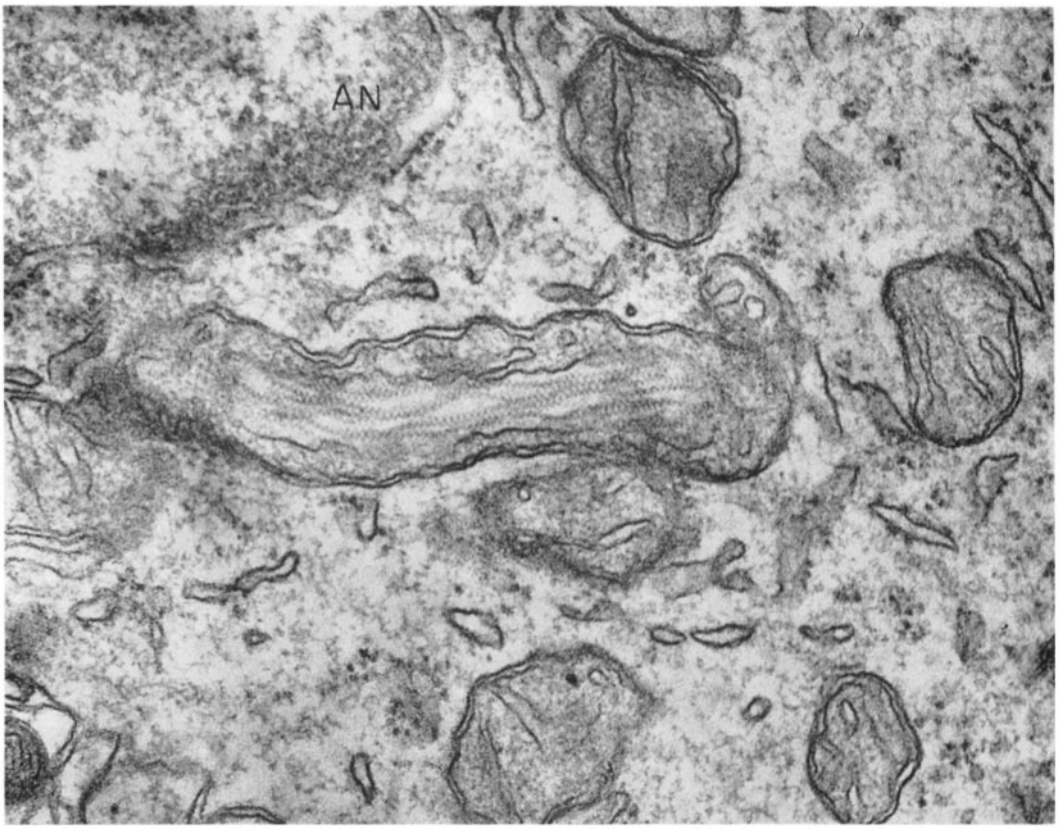

FIGURE 8 Detail from an astrocytic cell body showing a mitochondrion which lies near the nucleus and is almost completely occupied by a single, dilated intracristal space with helical filaments. × 50,000.

It should be discussed whether there is a relation between the intramitochondrial helical filaments and the bundles of cytoplasmic glial fibrils[1] and whether the latter might arise from the former. The glial fibrils seen with the electron microscope are known to form the ultrastructural basis for the fibrils visible in the light microscope. Early light microscopists observed that fibrilogenesis in astroglial cells begins at the periphery, *i.e.*, in the processes, and that the number of gliosomes (mitochondria) decreases while the fibrils are being formed. It was suggested that gliosomes participate in the formation of the glial fibrils (1, 7). That gliosomes are mitochondria is generally accepted by electron microscopists (6, 25). The present author, however, has seen no sign of disruption of the special mitochondria with liberation of filaments into the cytoplasm and does not believe that this may occur. Unfortunately, the electron microscope has given no information on the mode of glial fibrilogenesis so far, and studies of this process are wanting.

[1] In this account, in order to avoid confusion between cytoplasmic filaments and the helical filaments in mitochondria, I have preferred to term the filaments often seen in the astrocytic cytoplasm as "glial fibrils." Glial fibrils are 60 to 100 A thick, apparently straight, long units, arranged in bundles. Thus, they differ in shape and dimensions from the helical filaments seen in mitochondria.

Reports of unusual forms of mitochondria in glial cells now identifiable as astrocytes were mentioned in the Introduction. At present, a relationship between the mitochondria containing helical filaments and these types of mitochondria can not be completely excluded, but no intermediate forms have been seen. In particular, in certain special mitochondria described by Hartmann (11), Farquhar and Hartmann (6), Gray (9), and Mugnaini and Walberg (15), the fibrous content

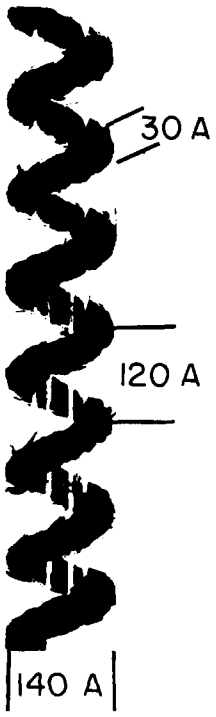

FIGURE 9 Model (made with a cord and a glass tube) of a helical filament with a right-hand screw axis. The filament is about 30 A thick, the total diameter of the helix is about 140 A, and the pitch is 120 A.

lies in the matrix and not intracristally as the helical filaments do. This may be an important difference, since it means that only one membrane is interposed between these helical filaments and the surrounding cytoplasm. This suggests that they may be exposed to a different enzymatic environment than are the structures in the matrix.

A few papers suggesting the existence of cytoplasmic DNA should be mentioned in the discussion of the mitochondrial helices seen in this study. Fibrous inclusions in mitochondrial matrix have been found in the ameba *Pelomyxa* (21), in mouse oocytes (23), and in the developing chick embryo (16). In the paper on *Pelomyxa* the existence of mitochondrial-nuclear relations was suggested. Nass and Nass (17) reviewed the evidence that these intramitochondrial filaments in several respects behave as DNA. Steinert (26) showed that, in the kinetoplast, two parts are seen, one being fibrous and Feulgen-positive, and the other of mitochondrial nature. Chèvremont *et al.* (5), in experimental studies on chicken embryo fibroblasts, described that mitochondria accumulate DNA and transport it to the nucleus. In view of these observations it could not be completely excluded that the helical filaments described in the present paper contain DNA,[2] and specific tests for the elucidation of this point should be carried out. The mitochondria with helical filaments, when present in the glial perikaryon, presented no evident relation to the nucleus other than spatial proximity.

Nothing conclusive can as yet be said about the specific role of the astroglial mitochondria-containing helical filaments. One contributing reason for this is that biochemical analyses of the enzymatic equipment of astrocytic mitochondria in general are lacking. Histochemical studies (particularly at the electron microscope level) are needed for a better understanding of the chemical nature of the filaments and will perhaps provide a more solid basis for a discussion of their functional significance.

Note Added in Proof: After this manuscript was submitted for publication, the author observed dilated intracristal spaces in a number of mitochondria from nerve cells in the rat corpus striatum, from glial cells in the brain of *Myxine glutinosa* L., and from human hepatocytes. These spaces contain a filamentous material. However, the typical helices seen in this study were not observed.

From these observations it appears that at least the dilatation of intracristal spaces and the presence within them of filamentous material is a phenomenon not limited to astrocytic mitochondria. The fact that only a part of the mitochondrial population in one cell presents such features may suggest that they are related to a particular phase in the life of the organelle.

[2] The helical filaments described within Feulgen-positive areas of ameba nuclei by Pappas and Brandt (21, 22) have a suggestive resemblance to the mitochondrial helices reported in this paper, even though they possess different dimensions.

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2nd Note Added in Proof: Helical filaments have now been found also in mitochondria from glutaraldehyde-fixed rat liver cells (O. Behnke, personal communication), and from osmium tetroxide-fixed myocardium cells of the chick embryo cultured *in vitro* (P. Buffa *et al.*, personal communication). In the former material the helices seem to be particularly numerous.