OBSERVATIONS ON PRISMATIC-TYPE MITOCHONDRIA WITHIN ASTROCYTES OF THE SYRIAN HAMSTER BRAIN

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ABSTRACT

This paper describes a special form of mitochondria which has been observed using the electron microscope in certain astrocytes of the Syrian hamster brain. The interior structural pattern of these mitochondria is characterized by the presence of numerous equilateral prismatic tubules in a highly regular hexagonal arrangement, instead of the customary foliate cristae. Such mitochondria have been designated as "prismatic type," in contradistinction to the more usually encountered crista and tubular types. The findings are compared with the occasional similar findings reported in the literature, and their possible functional meaning is briefly discussed.

INTRODUCTION

Most mitochondria in astrocytes of the mammalian central nervous system exhibit an ultrastructural organization that is prevalent in mitochondria of nearly all vertebrate cells, and of many invertebrate cells. This mitochondrial pattern is characterized by cristae which, as a rule, are transversely or obliquely oriented, and which have been interpreted as plate-like infoldings of the inner limiting membrane (18, 19). In addition to these customary mitochondria, extraordinary, long rod-shaped bodies with longitudinal striations of fairly regular spacing have been observed, on occasion, within the cytoplasm of marginal and intracortical astrocytes (9, 1, 23, 7, 15). These bodies have been thought to represent unusual forms of mitochondria. However, owing to the lack of adequate electron micrographs of sections in several planes, neither the actual internal structure nor the real nature of these organelles could be

hitherto clearly ascertained. It was, therefore, considered of value to examine both longitudinal and cross-sections of these questionable bodies in greater detail, in the hope of clarifying their interior three-dimensional configuration, and confirming or denying their mitochondrial identity.

MATERIAL AND METHODS

Brains of healthy adult Syrian hamsters (*Mesocricetus auratus*) were fixed and embedded by two different methods. Small pieces of tissue from the cerebral cortex were fixed immediately after removal in a buffered 1 per cent osmium tetroxide solution (17) for 3 to 4 hours at $+4°C$, dehydrated through a series of graded concentrations of ethanol, and embedded in a 9:1 butyl–methyl methacrylate mixture. As an alternative, whole coronal slices, 2 to 4 mm thick, were fixed for $1\frac{1}{2}$ hours in 6.5 per cent glutaraldehyde in 0.1 M phosphate buffer (22). Small tissue blocks were then cut out from many areas, washed,

and postfixed in 1 per cent osmium tetroxide for 1½ hours. Following ethanol dehydration this material was embedded in Vestopal W (13). Ultrathin sections were cut on either a Servall Porter-Blum I microtome or an LKB Ultratome using glass knives. Methacrylate sections were picked up on Formvar-coated copper grids, but Vestopal sections were mounted on uncoated grids, and were stained with lead citrate (21). All sections were examined in a Siemens Elmiskop I at initial magnifications of 2,000 to 40,000, utilizing an accelerating voltage of 80 kv and molybdene objective apertures of 20, 30, and 50 μ.

DESCRIPTION

The cytoplasm of astrocytes in the Syrian hamster brain sometimes contains large, sharply circumscribed bodies of relatively high density. These bodies are peculiar in that they have a distinctive interior ultrastructural pattern which is different from that of the customary mitochondria often closely associated with them in the same cell. These bodies are found within cytoplasmic extensions of marginal astrocytes which form most of the subpial and perivascular border zones of the cortical neuropil (Figs. 1, 3 to 5, and 7). Homologous structures may be very rarely identified within astrocytic processes elsewhere in the cerebral cortex and brain stem (Fig. 6). However, they have never been observed within the cytoplasm of neurons, oligodendrocytes, microglial cells, or ependymal cells.

These bodies are often very long, but when cut transversely are oval or round (Fig. 1), and one may assume that they possess a cylindrical or torpedo-like shape. It is remarkable that these bodies, as a rule, do not occur singularly, but often tend to form small accumulations within certain cytoplasmic areas (Figs. 1, 4, 6, and 7). It is difficult to decide whether such accumulations consist of several individual bodies, or represent one very long serpentine body, several segments of which have been cut and lie in the plane of a given section. If the latter is true, then the actual longitudinal dimension of these bodies is indeterminable. The largest profiles found are approximately 0.8 μ in diameter and up to 6 μ in length.

Longitudinal sections of these bodies show two parallel limiting membranes which are separated by a continuous space, about 70 to 120 A, the content of which has a very low density. The interior of the bodies contains a finely granular,

fairly opaque matrix in which numerous linear structures of varying density and thickness are observed. These linear structures are orientated approximately parallel to the longitudinal axis of the bodies, are 50 to 300 A apart, and often seem to be arranged in pairs. They extend for variable distances, and thus give the interior of the bodies a "pinstripe" pattern (Figs. 2 and 3). They may be interpreted as filaments, or as profiles of membranes which would thus be expected to enclose tubular or flattened spaces. Occasionally a pair of these linear structures is seen to bend outwards and then to become continuous with the inner limiting membrane (Fig. 3).

In cross-section, these bodies exhibit a fine structural organization of striking order and regularity. Within their finely granular matrix lie numerous membrane-bounded profiles with the shape of equilateral triangles (Figs. 4 to 8). These profiles are not randomly distributed within the matrix as would appear at first glance, but are arranged in a hexagonal lattice with a spacing of 470 to 480 A from centre to centre. However, these equilateral triangular profiles do not show any regular pattern in alignment of their angles and sides. The hexagonal lattice pattern is most precise in the central areas of the bodies. At the periphery of the latter the pattern of triangular profiles usually deviates a little. Severe deviations are very rarely found, and are probably due to the preparatory techniques. The equilateral triangular profiles are almost uniform in size except for the occasional larger one situated peripherally. Their sides, which are sometimes slightly concave, measure 250 to 330 A in length. The density of the contents of all the triangular profiles is like that of the light space separating the outer and inner limiting membranes. These two low density areas become confluent, in the case of some of the very marginally located triangular profiles, through membrane-bounded channels of varying width. This continuity is always established at the angle of the triangular profile that lies nearest to the inner limiting membrane (Fig. 8). Several sections of the same bodies at different levels show no variation in this described structural pattern.

In addition, one to three oval or round corpuscles of high density with a diameter of 300 to 500 A are found occasionally in the matrix of both longitudinally and cross-sectioned bodies (Figs. 2 to 4, and 7). They are very similar to the so called intramitochondrial granules which have

been described within mitochondria of many somatic cells in animals, as reviewed by Novikoff (16).

DISCUSSION

Longitudinal and cross-sections of the large, elongated bodies found within the cytoplasm of certain astrocytes in the Syrian hamster brain permit a three-dimensional reconstruction of the configuration of the bodies. The envelope is composed of two parallel membranes, the inner of which encloses a finely granular, moderately dense matrix containing numerous membrane-bounded tubules. The main characteristics of these tubules are: (a) an equilateral prismatic shape, (b) an orientation approximately parallel to the longitudinal axis of the body, and (c) an arrangement in a hexagonal lattice with a spacing of 470 to 480 A from centre to centre. It would appear that all the prismatic tubules have arisen from invaginations of the inner limiting membrane. The tubules originating from the poles of the elongated bodies are centrally placed, while those arising from their sides remain in a more peripheral location.

The contrast between the walls of the equilateral prismatic tubules and the matrix of the body is observed to be sharper and more uniform in cross-section than in longitudinal section. In the latter, the walls of the prismatic tubules are most often obliquely cut; less frequently they lie

vertical or parallel to the plane of the section. Consequently, the variety of positions presented to the electron beam results in a wide variety of contrasts. Furthermore, the average thickness of thin sections is about 500 A, and only slightly exceeds the 470 to 480 A distance between the axes of the prismatic tubules. Thus, in longitudinally cut bodies, part of one tubule can be expected to be superimposed upon that of another. This circumstance results in the "pinstripe" appearance of the bodies, and gives rise to difficulties in interpretation of their longitudinal submicroscopic structure.

We would emphasize that the basic ultrastructural features of the bodies described in this paper are identical after using osmium tetroxide or glutaraldehyde as the primary fixatives, and methacrylate or Vestopal W as the embedding media. In general, the obtaining of identical submicroscopic findings after the application of two different methods of fixation and embedding is good evidence that biological structures have been well preserved and closely approach the living state in their appearance. Nevertheless, an intriguing problem is whether the prismatic shape of the hexagonally arranged tubules exists in the living state, or whether it is the result of shrinkage during preparatory procedures. It is known that osmium tetroxide fixation causes shrinkage of tissue and that dehydration in alcohols produces further shrinkage. The partial re-


The material in Figs. 1, 3, 5, 7, and 8 was prefixed in glutaraldehyde, postfixed in osmium tetroxide, and embedded in Vestopal W; the sections were stained with lead citrate. The material in Figs. 2, 3, and 6 was fixed only in osmium tetroxide, and embedded in methacrylate; the sections were unstained.

Figure 1 Superficial cortex and leptomeninges of Syrian hamster brain. Within subpial astrocytic processes are seen longitudinally and cross-sectioned atypical mitochondria (PM). These are much larger and slightly denser than the majority of other mitochondria within the different cortical components. PC, pial cell; V, blood vessel; Er, erythrocyte. × 12,000.

Figures 2 and 3 Longitudinally cut atypical mitochondria (PM) within the cytoplasm of a perivascular astrocytic process and of a subpial astrocytic process. The outer and the inner limiting membranes are just discernible, but within the matrix the parallel linear structures can be followed for variable distances. In Fig. 3, pairs of these linear structures are sometimes seen to bend outwards and then to become continuous with the inner limiting membrane (arrows). Intramitochondrial granules (G) are clearly visible within the matrix. PC, pial cell; BM, basement membrane. Fig. 2, × 60,000; Fig. 3, × 80,000.

For figures, see following pages.
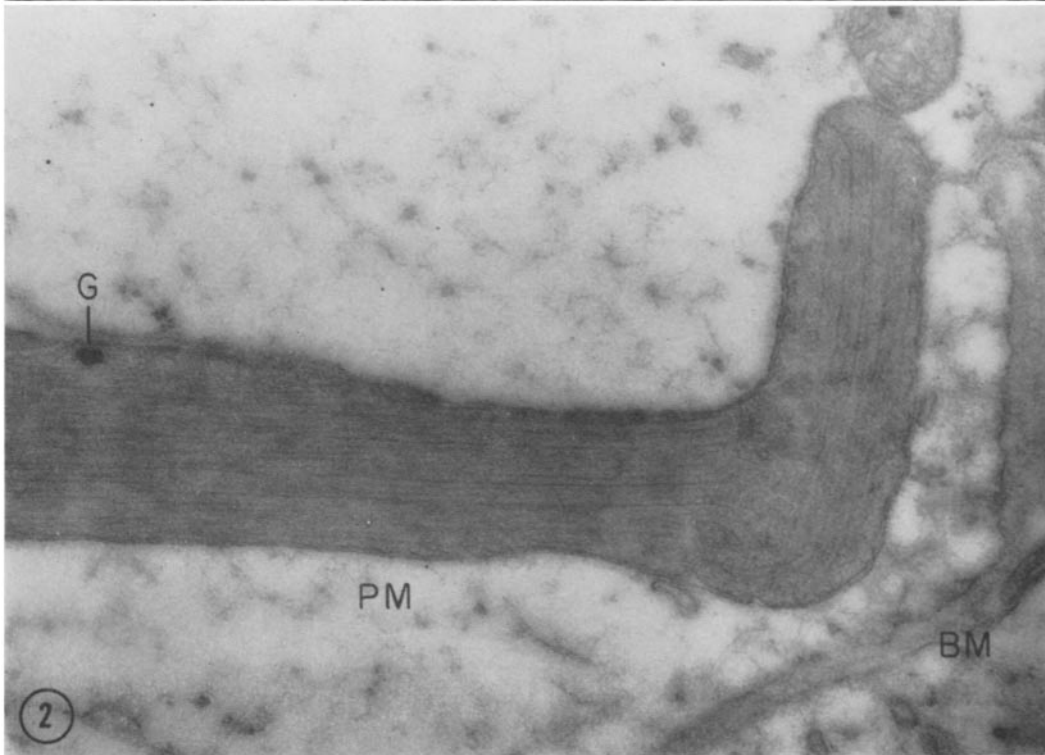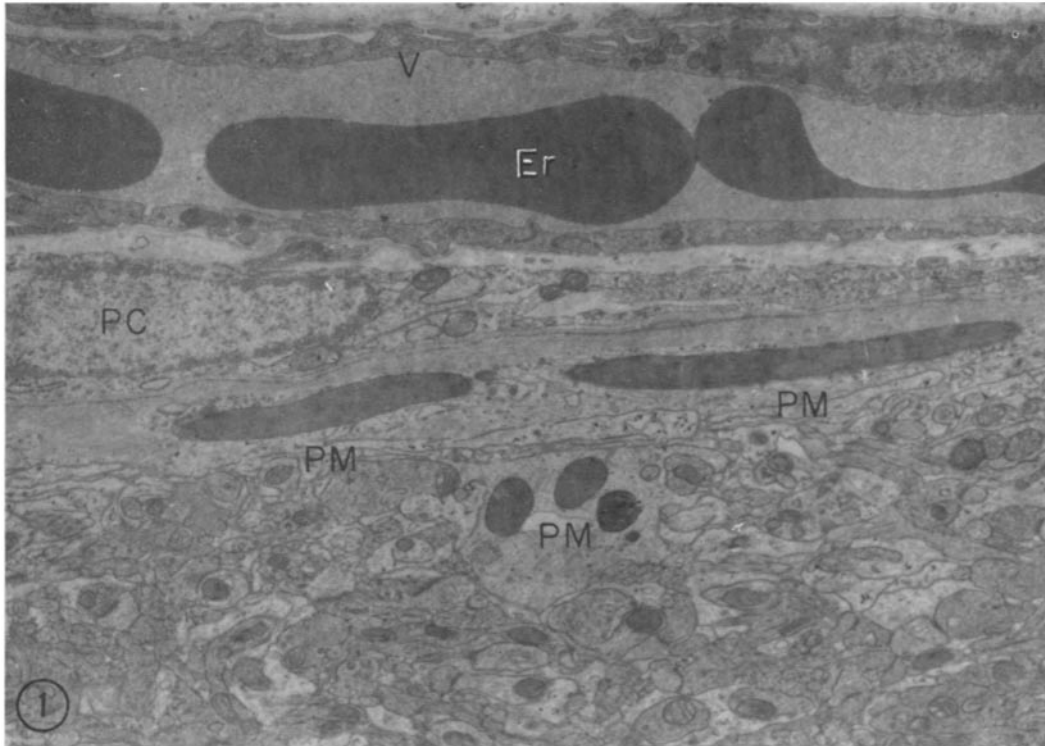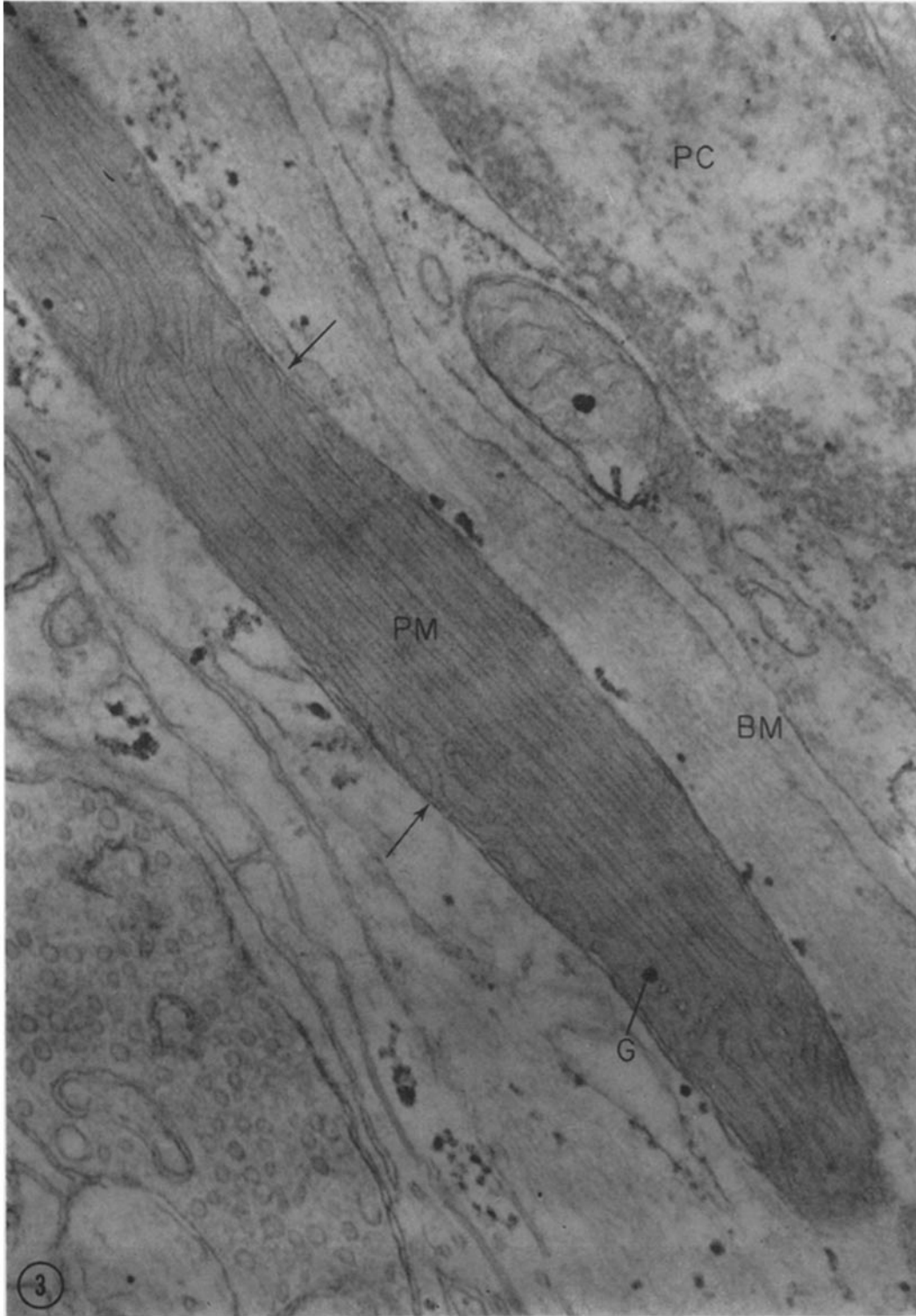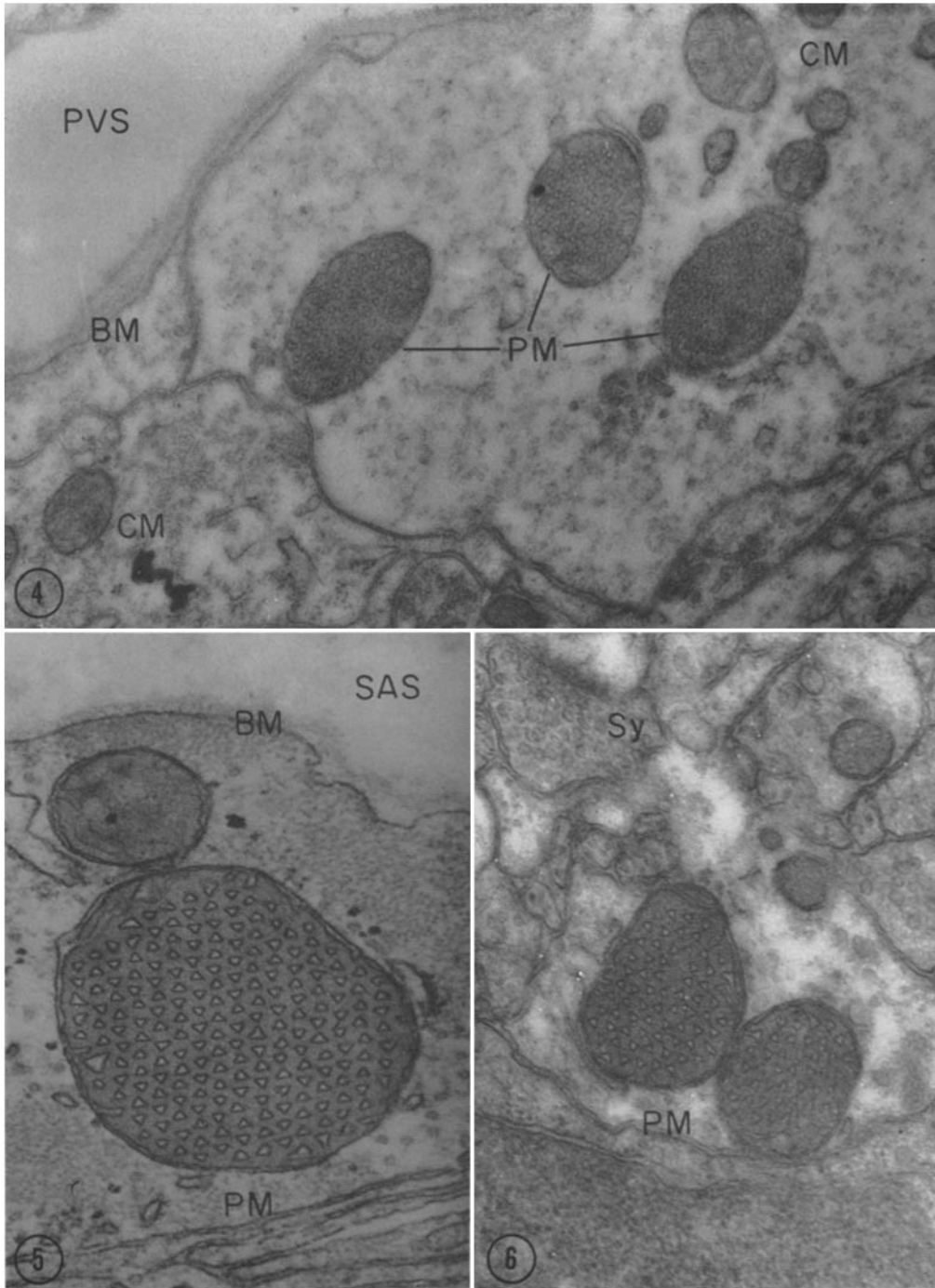
FIGURES 4, 5, and 6   Examples of cross-sectioned atypical mitochondria (*PM*) located within the cytoplasm of a perivascular astrocytic process (Fig. 4), of a subpial astrocytic process (Fig. 5) and of an astrocytic process elsewhere in the cortical neuropil (Fig. 6). Numerous triangular profiles are clearly visible within the matrix. In Fig. 4, the distinctive prismatic-type internal structure can be readily compared with the more commonly encountered crista-type (*CM*). *PVS*, perivascular space; *SAS*, subarachnoid space; *BM*, basement membrane, *Sy*, synapse. Fig. 4, × 40,000; Figs. 5 and 6, × 60,000.

expansion of tissue upon subsequent immersion in embedding media is largely compensated for by an additional shrinkage durin pgolymerization, sectioning, and exposure of sections to electron bombardment. For instance, combined x-ray diffraction and electron microscopic studies of nerve myelin sheaths have clearly demonstrated that the thickness of the repeating myelin units, as visualized in the electron microscope after application of the usual fixation and embedding techniques, is less than 70 per cent of their actual thickness deduced from x-ray diffraction patterns of fresh nerves (2–4). Similar reductions in size are produced in the highly regular, double hexagonal arrays of primary and secondary myofilaments in fibers of vertebrate striated muscle, as shown by comparison of electron microscope and x-ray diffraction data (11). These two examples indicate that in periodically or otherwise regularly ordered structure in biological systems such artificial diminutions in size are always symmetrical and do not alter the original basic patterns. Thus, both the hexagonal arrangement and the equilateral prismatic shape of the tubules within our described bodies may be presumed to exist in the living state. Regarding the shape of the prismatic tubules, another possibility must be considered, namely, whether they have been transformed from cylindrical structures by some obscure mechanism. One may imagine, for instance, that in a cross-section of one of these bodies, at any given level, fine fibrous protein filaments lying within the matrix and inserting into the wall of each tubule at three definite, equidistant points may form a strutting framework cross-linking the individual tubules. Preparatory procedures might induce a shortening of such imaginary filaments, as well as a contraction of the wall of each hypothetical cylinder. The filaments at the three points of insertion into the walls of these cylinders would not allow a symmetric diminution of the latter, but would cause a distortion resulting in the prismatic configurations observed by us. It should be mentioned here that, in the A, I, and Z bands of insect flight muscle, each of the hexagonally spaced myofilaments is connected radially with its six surrounding neighbours by thin filamentous bridges (10). All attempts made to detect such supporting elements in the interior of the bodies described by us were unsuccessful. On the other hand, some organization within the matrix does appear in the form

of fine dots which are most pronounced after glutaraldehyde fixation. In addition, the prismatic tubules themselves lack any definite regular pattern as regards alignment of their edges or sides. This indicates, perhaps, some freedom of rotation within the matrix, rather than a rigid tubular arrangement which would result from the presence of cross-linking filaments. Therefore, the question concerning the shape of these tubules in the living state remains unanswered, but we believe that they exist originally in the observed prismatic form.

In order to discuss the possible mitochondrial nature of these unusual bodies within the astrocytic cytoplasm, it is necessary to recall the essential submicroscopic features of mitochondria. In his original communications on mitochondrial ultrastructure, Palade (18, 19) noted the existence of two parallel limiting membranes, and stated that the inner one of these is infolded. Many subsequent investigations have corroborated these findings. In most cells the invaginations of the inner mitochondrial membrane form foliate cristae, whereas in protozoa and in several metazoan cells they have been shown to be either straight cylinders or undulating villous projections. The considerable literature on the subject has been extensively reviewed by Miller (14) and by Novikoff (16).

There is no doubt that the bodies described in this paper fit Palade's general basic model of the mitochondria with respect to the envelope and interior structure. Consequently, from a morphological point of view, the mitochondrial identity of the bodies can be affirmed. However, though these bodies are in keeping with Palade's concept of the fundamental mitochondrial configuration, their internal structural pattern is exceptional in that it consists of equilateral prismatic tubules in hexagonal arrangement instead of the customary plate-like cristae. It is, therefore, reasonable to consider them as representing a special form of mitochondria.

In the literature, mitochondria are sometimes specified either as crista type or as tubular type, according to whether the infoldings of their inner limiting membranes are foliate or cylindrical (6, 24). We would suggest that the organelles described here constitute a third type, which we wish to designate as the prismatic type. This term, we feel, suitably encompasses the basic characteristics of their distinctive interior structure

which does not resemble that of either the crista or the tubular types.

We wish to emphasize that prismatic-type mitochondria represent only a very small fraction of the entire mitochondrial population within astrocytes of the Syrian hamster brain. More precise information concerning their actual frequency or their approximate ratio to the crista-type mitochondria cannot be given. Our electron microscope studies on the fine structure of the mammalian central nervous system have been performed on Syrian hamster brains, and with diligent search such mitochondria could often, but not always, be identified.

Atypical mitochondria, resembling those within the astrocytic cytoplasm, were described recently in the cricothyroid muscle of the bat by Revel, Fawcett, and Philpott (20). Within the interior of these, a sharply circumscribed area was occupied by prismatic tubules. In contrast to our findings, however, these tubules were completely surrounded by the usual plate-like cristae, and end-to-end continuities of the former with the latter could often be recognized. Further examples of a departure from the customary mitochondrial ultrastructure have been reported in various vertebrate cells by other authors. However, these are much less comparable with our findings than that noted by Revel and his associates. For instance, within neuroglial cells of the cochlear grey in the lizard brain, Gray (8) described peculiar mitochondria whose interior showed longitudinally orientated cylindrical tubules with a regular hexagonal spacing. A finding similar to that of Gray was recorded as an incidental observation

two years earlier by Fleischhauer (5) in the ependymal "tanycytes" of the blind-worm (*Anguis fragilis*).

The possible functional significance of these prismatic-type mitochondria must be touched upon briefly. From a qualitative point of view, mitochondria in all kinds of aerobic organisms probably possess a common basic enzymatic equipment. However, biochemical studies have shown that the individual enzymes which constitute the respiratory multienzyme systems vary quantitatively from species to species, and, even in the same species, from one organ to another (16, 12). The results of comparative electron microscope investigations indicate that such variations in the quantities of mitochondrial enzymes can be correlated mainly with differences in the size of mitochondria, and with differences in the ratio of their total inner membrane surface to their total matrix volume (24). Moreover, several biochemical findings have given rise to the belief that mitochondria serve not only for nonspecific intracellular energy transformation, but also play an intimate role in highly specific intracellular metabolic activities (12). It is still unknown whether such specific functions of mitochondria are associated with certain characteristics in their fine structure. We are also unaware of any convincing biochemical or topochemical observations which would suggest the existence of functionally different kinds of mitochondria within one and the same cell. Consequently, we can only speculate that the distinctive ultrastructural organization of the interior of the prismatic-type mitochondria may be related to obscure metabolic

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Fig. 7   Pericapillary astrocytic processes containing atypical mitochondria. The mitochondrion in almost perfect cross-section (*PM*₁) shows the triangular profiles within its interior, the other obliquely cut (*PM*₂) shows the transition between the triangles of *PM*₁ and the parallel paired structures within the matrix of the longitudinally sectioned organelles of Figs. 2 and 3. Within the matrix of *PM*₂ is an unusual intramitochondrial granule (*G*) exhibiting a dense periphery and a light centre. *CL*, capillary lumen. × 60,000.

FIGURE 8   Higher resolution reveals the almost equilateral configuration of the triangular profiles within the matrix of a transversely cut atypical mitochondrion. Occasional peripheral profiles appear larger and less regular in shape, and are seen to be continuous with the interspace separating the outer and inner limiting membranes. On careful viewing, the described hexagonal arrangement of the equilateral triangles becomes apparent (compare with the Fig. 9). × 120,000.
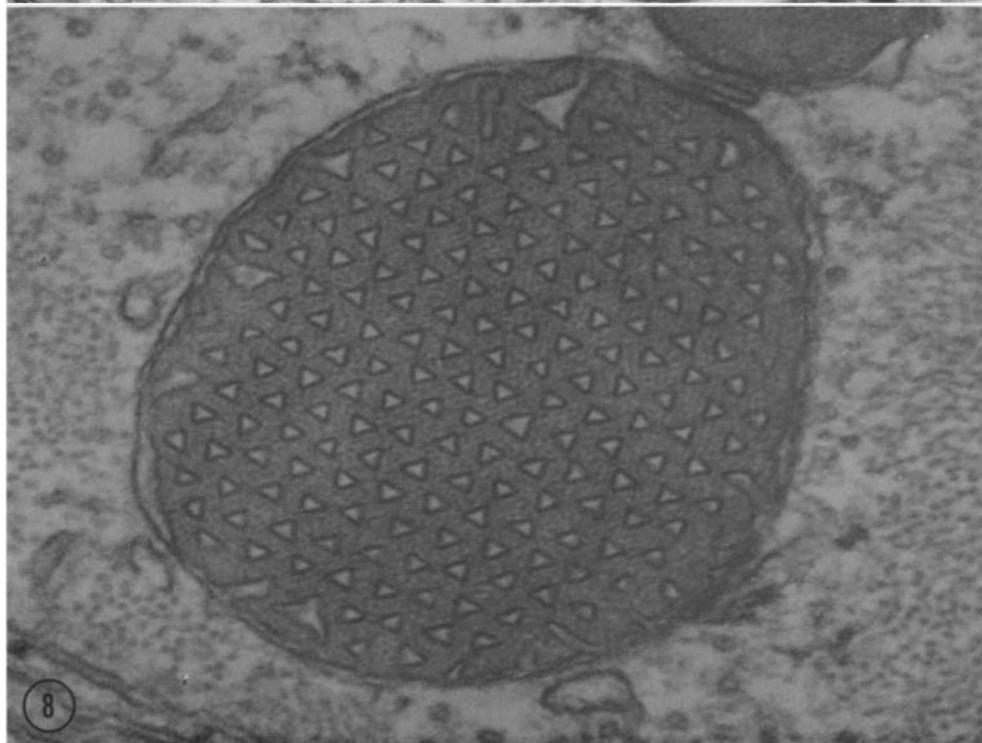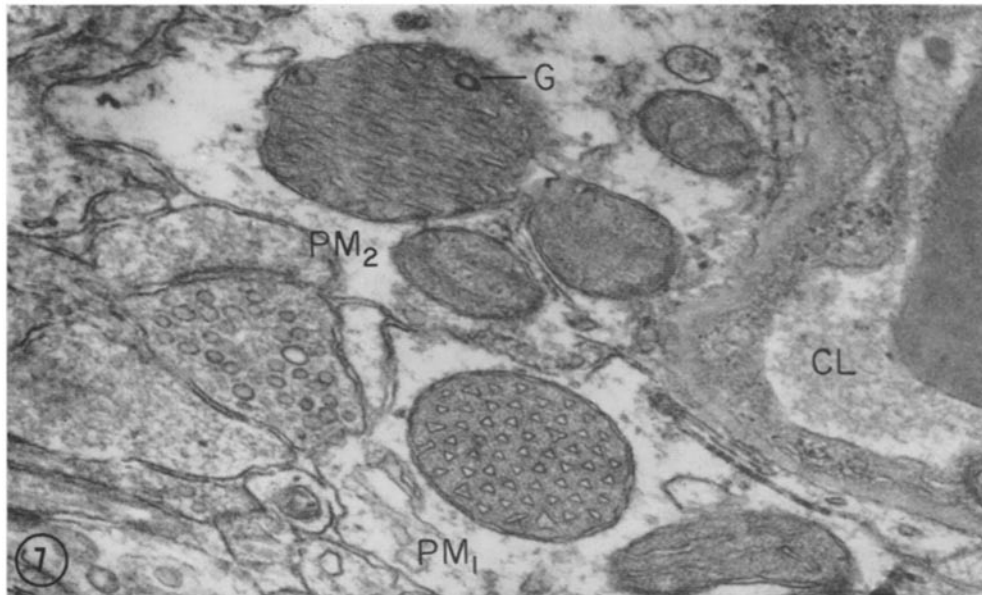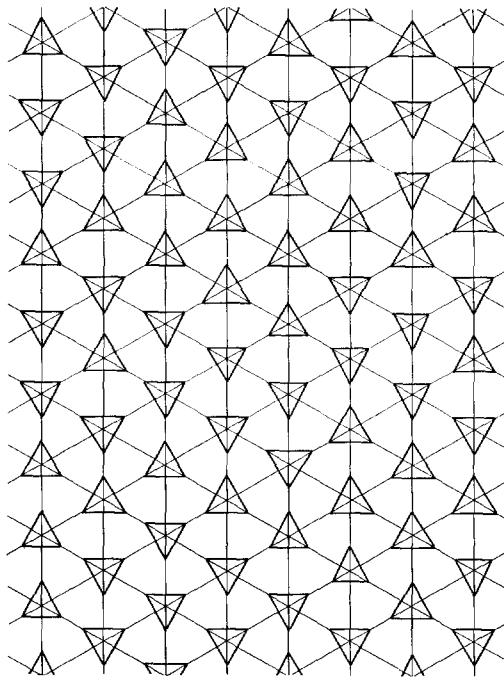
FIGURE 9 This shows the highly regular hexagonal spacing of the equilateral triangular profiles within the interior of cross-sectioned atypical mitochondria. The thin lines passing through the centre points of each triangular profile indicate their arrangement in three series of parallel, equidistant, straight rows intersecting each other at angles of 60° or 120°.

properties. If this is true, then these prismatic-type mitochondria differ, both morphologically and functionally, from mitochondria exhibiting the usual foliate cristae.

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