Brit. J. Ophthal. (1966) **50**, 325

PART II. ULTRASTRUCTURAL CHANGES IN FOCAL RETINAL ISCHAEMIA

BY

MANOUCHER SHAKIB AND NORMAN ASHTON

INDEX—PART II

THE technique of experimental embolization has particular value for an electron microscopical study since the lesions can be inflicted at a given moment and the evolution of pathological changes traced in a specimen wherein perfect fixation can be obtained and the affected area precisely located (Fig. 1). The observations are thus surely attributable to simple ischaemia, and can be interpreted with an exact knowledge of their time relationships. In this section we report our detailed findings, both by light and electron microscopy, on the retinal material from the pig experiments already described in Part I of this work.

Methods

The enucleated eye was immediately divided into two halves by coronal section through the ora serrata; the vitreous was gently removed and the posterior half of the eye immersed in chilled 1 per cent. isotonic veronal buffered osmium tetroxide. In order to allow a better penetration by the fixative and at the same time to keep the retina flat, a thin spatula was introduced between the choroid and the retina producing partial retinal separation (a totally separated retina becomes folded and consequently localization of the lesion is then difficult, if not impossible). Fixation was carried out for two hours in fresh changes of fixative at 4°C and the specimens were dehydrated in graded concentrations of alcohol.

Pieces of retina measuring 4–6 mm. in diameter, which included the area of the lesion, were excised for embedding in Epon. When the lesion had been clearly visible by ophthalmoscopy and recorded by fundus photography, it was readily found under the dissecting microscope, but in more prolonged experiments when a slight granularity of the retina was the only finding on fundoscopy, it was practically impossible to detect the lesion under the microscope unless fundus photographs or drawings were used in the same manner as for localization of retinal holes in detachment surgery. Here the landmarks were blood vessels and the shining glass spheres in the lumina of the arterioles. In this way the exact area of embolized retina could be selected and excised.

Sections 1 μ thick were cut from the whole block with a Huxley microtome, and stained with 1 per cent. alkaline toluidine blue, studied by light microscopy, and photographed. Thin sections were cut from the selected area of the block and stained with uranyl acetate followed by lead citrate and viewed with an A.E.I. EM6 electron microscope.

Findings

Light Microscopy

One-hour Specimen.—Light microscopical studies of the osmium-fixed Epon-embedded sections at low magnification showed a well demarcated zone of elongated oval vacuoles throughout the nerve fibre layer (Fig. 2). Under high power the vacuolation appeared to be due to swelling of the individual nerve fibres, some of which were denser than normal and appeared compressed by neighbouring swollen fibres. At the borders of the lesions the nerve fibres, although of normal size, showed an increased granularity. In the region of swelling some of the ganglion cells showed clear areas in their cytoplasm and the chromatin pattern of their nuclei was more pronounced, while the inner molecular layer was more vesicular than normal. Clear cells with a pronounced chromatin pattern could also be seen in the inner nuclear layer.

Five-hour Specimen.—Swelling of the nerve fibres was now more severe, giving rise to bulging of the internal limiting membrane, and on either side of the swollen area there was a definite margin of less expanded fibres containing densely staining granules, which were found to be mitochondria (Figs 3 and 4). The changes in the ganglion cells were more advanced and all those affected showed granular nuclear staining; the cell membranes were still intact (Fig. 5). The inner plexiform layer was markedly vesicular and in addition there were areas which had stained intensely. Clear cells, some with dark nuclei and others with pale nuclei, were seen in the inner nuclear layer (Fig. 5). In areas where swelling of the nerve fibre was most extensive some of the associated capillaries had slit-like lumina, but no completely closed capillaries were seen.

Twenty-four-hour Specimen.—At this stage the affected area still remained swollen and some of the nerve fibres showed expansions which contained homogeneous or coarse granular material. Some of the ganglion cells were now completely degenerate, being represented by cystic cavities devoid of cytoplasm and with dark irregular nuclei on one side of the cells (Fig. 6). The inner molecular layer appeared more vesicular than that of the five-hour specimen, and between the vesicles there were dark-staining granules. In the inner nuclear layer vacuolated cells with dark nuclei were seen amongst the less damaged or even normal cells.

Three-day Specimen.—There was now little bulging of the internal limiting membrane and the nerve fibre layer no longer showed the vesicles which were such a prominent feature in previous specimens; they were now replaced by round or oval bodies about the size of ganglion cells, containing closely packed basophilic granules with occasional pale segments. These bodies appeared to be expansions of the nerve fibres (Figs 7 and 8). The ganglion cell layer showed more advanced atrophy and the hyperchromatic granularity of the inner molecular layer was more prominent. The cells of the inner nuclear layer showed some loss of nuclei and swelling and pallor of the cytoplasm. Scattered throughout this layer there were numerous dark-staining cells.

Seven-day Specimen.—The round or oval bodies in the nerve fibre layer now appeared denser or in some cases vesicular (Fig. 9). A number of macrophages were scattered throughout all layers of the affected area; these cells, characterized by an irregular nucleus with densely staining debris in the cytoplasm, were usually found near blood vessels and often clustered around degenerate ganglion cells. The inner molecular layer showed some areas of atrophy and the inner nuclear layer appeared similar to the previous specimens.

Fourteen-day Specimen.—The atrophic nerve fibre layer contained a few round or oval bodies which were smaller and more darkly staining than those present earlier. The macrophages had increased in number and were usually seen around the vessels (Fig. 10). No vacuolation was present in this layer. Many of the damaged ganglion cells had disappeared and in this region macrophages containing abundant basophilic granules in the cytoplasm were seen; they were also present in the inner nuclear layer and the atrophic inner molecular layer.

Twenty-five-day Specimen.—The appearances at this stage were striking. The retina, in the area of the lesion, was extremely atrophic and disorganized in its inner layers. The majority of the nerve fibres were completely replaced by glial cells and those remaining appeared normal. The ganglion cells and inner plexiform layer were absent and were also replaced by glial cells among which there were a few macrophages (Fig. 11). The normal architecture of the outer plexiform layer was lost.

Haemorrhages

In addition to the pathological changes already described, erythrocytes, leucocytes, and fibrogranular deposits were scattered throughout the affected area, but these elements were usually localized in the inner layer of the retina (Fig. 12). When the haemorrhage was extensive, rupture of the internal limiting membrane with some loss of the innermost part of the retina resulted.

Electron Microscopy

Although the intensity of the degenerative changes found on electron microscopy varied in the same lesion, in the overall study they appeared to show a definite sequence of events.

One-hour Specimen

(*i*) *Nerve Fibre Layer.*—Enormously swollen nerve fibres with intact axonal membranes were scattered amongst many axons which were either normal or compressed by the swelling (Fig. 13). The enlarged segments measured up to 10 μ in diameter and were filled with a fine granular material containing sparse neurofilaments, a few microvesicles, dense bodies, and distended mitochondria (Fig. 14); on the other hand, some axons with normal diameter contained a greater proportion of dense bodies, membrane-bound vesicles, and apparently normal mitochondria. There was no extracellular fluid or fibrin. The affected axons were usually found near the ganglion cells.

(*ii*) *Ganglion Cells* (Fig. 15).—In some ganglion cells the cytoplasmic organelles were abnormal and were not present near the axonal hillock. Mitochondria were sometimes distended and often irregular in shape; their matrix was pale and their cristae present only at the periphery. In a few of these swollen mitochondria the cristae were broken up into vesicles, while others contained fine granular material. Alternatively, some mitochondria appeared contracted with an electron-dense matrix and almost indiscernible cristae. Rough-surfaced endoplasmic reticulum was reduced or completely lost on the side of the axonal hillock and the ribosomal particles appeared scattered throughout the cytoplasm. The smooth-surfaced endoplasmic reticulum showed a parallel increase. Occasionally, round concentric membranous dense bodies were present. The Golgi system was more pronounced than normal. In the nuclei there was a suggestion of chromatin aggregation, particularly near the nuclear membranes which were beginning to separate widely.

(*iii*) *Inner Plexiform Layer.*—A few of the axons and dendrites were expanded and contained swollen mitochondria (Fig. 16).

(*iv*) *Inner Nuclear Layer.*—Many of the cells of this layer appeared depleted of cytoplasmic structures and frequently the mitochondria were swollen with short broken cristae and a pale matrix. The cytoplasm often appeared electron-lucent with occasional ribosomes scattered amongst fine granular material; in other cells the ribosomes were in clumps and the Golgi complex and the endoplasmic reticulum were enlarged and vacuolated (Fig. 17). Few cells showed any nuclear changes without abnormality of the cytoplasm. Where these changes did occur the chromatin was aggregated into clusters and the nuclear membranes became widely separated in places. The capillaries were usually normal, but in areas where swelling of the surrounding retina was pronounced they appeared to be somewhat compressed and contained no red cells in their lumen, but in no case were they compressed to the point of complete closure.

(*v*) *Outer Plexiform Layer.*—Dendritic swelling was also found in this layer.

Five-hour Specimen

(*i*) *Nerve Fibre Layer.*—Many enlarged or swollen axons (similar to those of the one-hour specimen) filled with mitochondria, membrane-bound microvesicles and tubules, dense bodies and neurofilaments were interspersed between axons with normal diameters (Fig. 18). Mitochondria varied greatly in size and shape and measured on average between 0·15 and 0·5 μ in width. Some were contracted and elongated with a dense matrix, but others were swollen with pale mitochondrial contents and short cristae. Membrane-bound microvesicles and tubules, measuring on average 0·5–8 mμ in diameter, constituted the predominant components of the affected axons. The tubular profiles were lined by a continuous limiting membrane and the microvesicles are probably cross-sections of these tubules. Several types of dense bodies were recognizable; some consisted of densely packed concentric membranous structures, while others appeared as circular or oval electron-opaque structures of variable size. Dense bodies intermediate between these two types and measuring on average between 2 mμ and 0·3 μ were also discernible (Fig. 19). Neurofilaments were indistinct, short, thinner than normal, and were the least significant component of the damaged axon. Only a few poorly orientated bundles were found amongst intracytoplasmic structures. Swelling of the axoplasm was evident at focal areas along the length of the axons, although the axolemma appeared intact.

(*ii*) *Ganglion Cell Layer.*—Depletion of the cytoplasmic organelles on the axon hillock side of the cell was typical. The mitochondria were generally swollen and the endoplasmic reticulum dilated. On the whole the cytoplasm appeared pale, electron-lucent, and granular. In the nucleus the aggregation of the chromatin into clumps was more evident and adherence to the nuclear membrane was marked (Fig. 19).

(*iii*) *Inner Plexiform Layer.*—Distended or condensed dendrites and axons were commonly found; most of their mitochondria were swollen but some were electron dense, round or irregular in shape. In the swollen axons and dendrites, which contained a rather sparse finer granular material, a few vesicles were seen (Fig. 20). Müller fibres, where they could be distinguished, appeared to be shrunken.

(*iv*) *Inner Nuclear Layer.*—Degeneration of the affected cells was more advanced, and some appeared almost empty with a fragmented cytoplasmic matrix, a few

disintegrated mitochondria, and vacuoles. The nuclear chromatin was densely clumped and the nuclear membrane had partially disappeared (Fig. 21).

(*v*) *Outer Plexiform Layer.*—The changes in the neuroglial extensions of the inner layer were similar to those of the inner plexiform layer. In some sections one or two macrophages were encountered.

Twenty-four-hour Specimen

(*i*) *Nerve Fibre Layer.*—A striking feature at this stage was the large number of enormous, grossly pathological axons, about 5 μ in diameter (Fig. 22). The same structural components as had been seen in earlier stages were discernible in these fibres, but several new elements had also appeared. There was a reduction in the number of membrane-bound vesicles, but the number and size of the dense bodies had increased. The neurofilaments were more prominent. The newly formed elements consisted of microvesicular bodies and large membrane inclusion whorls. Occasionally, vesicles were enveloped by one or two layers of membrane to form microvesicular bodies. The membrane inclusion whorls appeared to consist of concentric arrays of smooth-surfaced membranes enclosing mitochondria, dense bodies, vesicles, and ribosomal particles. Scattered throughout this layer were large membrane-bound vacuoles which appeared to be the remnants of enormously expanded axons (Fig. 23). The surrounding membrane of these vacuoles seemed relatively normal.

(*ii*) *Ganglion Cell Layer.*—Many of the cells now had a ruptured plasma membrane and contained only empty vacuoles, vesicles, and dense bodies scattered in a fragmented cytoplasm. The nucleoplasm showed coarse granules which were unevenly dispersed and of medium electron density. A number of ganglion cells were actually engulfed by macrophages (Fig. 24).

(*iii*) *Inner Plexiform Layer.*—The Müller fibres contained dense, homogeneous, cytoplasmic droplets with scalloped margins and no limiting membrane, i.e., lipid droplets. Structures limited by a membrane consisting of one or two uneven, wrinkled, dark layers, which enclosed a compact coiled membranous core not unlike mitochondrial cristae, were present in both glial and neural components of this layer. Some of the swollen dendrites or axons appeared as vacuoles with broken membranes, which occasionally contained fragmented mitochondria and a collection of granular material at one side; other axons or dendrites contained dense cytoplasmic material. Throughout the neuropil of this layer many of the mitochondria were enlarged and had irregular outlines; in some the internal structure could not be distinguished, while in others the cristae had broken into microvesicles (Fig. 25).

(*iv*) *Inner Nuclear Layer.*—Clear cells were frequently found in which the nuclear and cytoplasmic membranes were dense and disrupted in many places and similar to those of the five-hour specimen. Some cells had dense cytoplasmic organelles and clumps of ribosomal particles, while in others the cytoplasmic matrix and organelles had disintegrated (Fig. 26). In some Müller cells the endoplasmic reticulum was distended while the outer nuclear membrane was seen to be intact and continuous with the membrane of the endoplasmic reticulum.

(*v*) *Outer Plexiform Layer.*—Similar changes had occurred as in the inner plexiform layer, and no alteration was noticed in the rod and cone pedicles.

Three-day Specimen

(*i*) *Nerve Fibre Layer.*—This layer was now characterized by the large number of grossly pathological swollen axons. Several types were distinguishable, possibly representing different patterns of the same degenerative process; some contained dense masses of neurofilaments which were either arranged in spirals or followed an irregular course and were often distorted by the other axonal contents (Fig. 27). In other axons the mitochondria were the most prominent component, and were mostly elongated and contracted with an increased electron density of their matrix (Fig. 28). A lesser number of damaged axons were typified by swollen mitochondria which appeared as vacuoles containing small vesicles (Fig. 29). Many of the swollen axons now contained a greater number of elaborate membranous whorls (Fig. 30). In some a spiral arrangement enveloping several groups of cytoplasmic elements was found; in others the number of concentric layers had increased and between these layers several elongated, irregular, membrane-bound vesicles containing granular material were seen. These whorls greatly varied in size and shape, sometimes measuring up to 2 μ or more (Figs 31 and 32). Occasionally they consisted of compressed membranes devoid of inclusion particles (Fig. 33). In all axons a great reduction in the number of membrane-bound microvesicles was apparent and their membranes appeared denser and thicker. The number of dense bodies had increased and they usually measured between 2 mμ and 60 mμ and varied greatly in shape and electron density (Fig. 28). Some were closely associated with the mitochondria and were round or oval with dark, thick, outer membranes enveloping compact coiled membranous structures; these could have been derived from mitochondria (Fig. 32). Others were irregular in shape consisting of concentric wrinkled membranes, limiting a denser central core. Most of the latter elements were electron-opaque without any internal structures (Fig. 31). In this layer the glial cells and their fibres occasionally showed signs of reactivation and contained a large amount of ribosomes, free or in association with endoplasmic reticulum (Fig. 34). They especially contained long irregular masses of amorphous material surrounded by a dense rim which represented intracytoplasmic lipid accumulation.

(*ii*) *Ganglion Cell Layer.*—Most of the degenerating cells appeared as vacuoles with fragmented nuclear and cytoplasmic material aggregated on one side. Macrophages were seen engulfing these necrotic cells.

(*iii*) *Inner Plexiform Layer.*—Atrophy of the neuropil and a proportional increase in the amount of glial tissue were found in this layer. Swollen axons and dendrites were rarely encountered, but many with dense cytoplasmic matrices and internal structures similar to those of the twenty-four-hour specimen were seen. In a few of these elements the cytoplasmic density had increased. The glia was electron-lucent and contained vesicles and aggregates of dense granular material; macrophages containing ingested material were distributed near the capillaries (Fig. 35).

(*iv*) *Inner Nuclear Layer.*—In the necrotic cells the fragmented cytoplasm was aggregated into osmiophilic material with dense bodies, vacuoles, and remnants of disintegrated mitochondria. The nucleoplasmic substance was coarser and the chromatin clumps were more electron-dense. The macrophages were seen in close association with these fragmented cells.

(*v*) *Outer Plexiform Layer.*—The changes in this layer were similar to those of the inner plexiform layer (Fig. 36).

Seven-day Specimen

(*i*) *Nerve Fibre Layer.*—The large swollen axons were now further altered; in some the elements were condensed and had indistinct margins. The mitochondria were compact, highly electron-dense, and their cristae could be recognized only under high magnification as contracted disintegrated structures. The membrane whorls were now condensed and usually their cytoplasmic inclusions could not be distinguished (Fig. 37). Areas of the membranes had often fused leaving electron-transparent spaces between them (Fig. 33). In other cases membranous whorls, mitochondria, and dense bodies appeared to be surrounded by a clear rim. In many of the pathological axons condensation of the axoplasmic elements mentioned above had progressed to such a degree that the whole structure was filled with coarsely granular electron-dense masses (Fig. 38). Other axons were characterized by prominent arrays of interwoven, dense, thick neurofilaments arranged in a finger-print pattern (Fig. 39). Some axons now appeared fragmented and the axonal membrane was often completely disintegrated. Only clumps of irregular, coarsely granular material and the remnants of membranous whorls, dense bodies, mitochondria, etc., remained (Fig. 37). In addition to these prominent pathological axons, a few unevenly electron-opaque axons were present. The abnormal degenerating axons were either surrounded by neuroglia (Fig. 37) or were engulfed by macrophages and were undergoing active phagocytosis (Fig. 40). In fact it was in this seven-day specimen that so much phagocytic activity in the nerve fibre layer was first noticed. Aggregations of intracytoplasmic material similar in appearance to that of the degenerating neurons were occasionally encountered in the glia (astrocytes). Some of the degenerating neurons were completely trapped in the cytoplasm of the glial cells and appeared as irregular electron-opaque masses. The axoplasm was often distributed and scattered loosely in the glial cells as vacuoles and irregular bodies of variable density. These glial cells often contained a number of lipid droplets and prominent nuclei suggesting phagocytic activity (Fig. 41).

(*ii*) *Ganglion Cell Layer.*—The degenerated ganglion cells were almost completely phagocytosed and replaced by glial elements. Cells with dilated endoplasmic reticulum and a large amount of ribosomes were seen particularly close to the blood vessels. These appeared to be reactive glial cells.

(*iii*) *Inner Plexiform Layer.*—The general appearance of this layer resembled that of the three-day specimen, but there was greater atrophy of the neuropil and the macrophage activity was more evident.

(*iv*) *Inner Nuclear Layer.*—Necrotic cells were now being replaced by glial tissue.

(*v*) *Outer Plexiform Layer.*—No further significant changes.

Fourteen-day Specimen

(*i*) *Nerve Fibre Layer.*—A striking feature was the great reduction in the number of nerve fibres accompanied by an obvious proliferation and extension of the glial processes (Fig. 42). Most of the remaining axons appeared either normal or in various stages of degeneration typical of earlier specimens. The axons in the final stages of disintegration were almost entirely engulfed by macrophages which were typically found adjacent to blood vessels (Fig. 43). The remnants of the axons inside them

appeared as a fairly homogeneous mass of medium electron-dense material, often containing dense bodies, linear components, and large vacuoles which appeared to be partially or completely filled with a lipid substance (Fig. 44). Under higher magnification the phagocytosed mass was seen to consist of loosely distributed, coarsely granular material harbouring a number of layered membranous structures, which were somewhat similar to myelin, consisting of parallel alternate dark and light layers, sometimes up to nine in number (Fig. 44). In these lesions the reactive glial cells were more prominent and their number had increased. These cells were characterized by a large amount of widely dilated rough-surfaced endoplasmic reticulum containing finely reticulated material. There was an increase in the number of mitochondria, and free ribosomes were much more prominent and sometimes were aggregated into rosettes. Scattered throughout the cytoplasm were electron-dense, oval or round membrane-bound bodies and lipid vacuoles. The nucleoplasm of these cells exhibited granular compact chromatin, often aggregated at the nuclear membrane and contained a nucleolus. The cell processes showed tightly packed tonofilaments. Very distinct junctional complexes between the glia were sometimes encountered (Fig. 42).

(*ii*) *Ganglion Cell Layer.*—The ganglion cells were replaced by glial elements similar to those described above, and macrophages with the cytological characteristics already mentioned were distributed throughout this layer. The few ganglion cells which survived appeared normal.

(*iii*) *Inner Plexiform Layer.*—Atrophy of this layer was advanced and macrophages were present.

(*iv*) *Inner Nuclear Layer.*—Macrophages were still present in this layer and many glial cells had replaced the normal cellular structures.

(*v*) *Outer Plexiform Layer.*—The inner part of this layer was more atrophic and a few macrophages were still present.

Twenty-five-day Specimen

(*i*) *Inner Layers.*—The retinal architecture was now so altered that the individual layers could no longer be recognized. The normal structure had been converted into a compact, complexly interwoven meshwork of glia, in which some macrophages, neural elements, and blood vessels were interspersed. A few nerve fibres, particularly those with numerous neurofilaments described in previous specimens, were found in the superficial layers. In longitudinal sections the axons appeared to follow an extremely tortuous course. The large dense phagocytic masses which were present in the cytoplasm of macrophages of earlier specimens had been replaced by a number of relatively smaller masses of varying size, shape, and electron density (Fig. 45). These macrophages were situated close to the blood vessels. Changes in the reactive glia had progressed little in comparison with the previous specimen; the number of microvesicles had increased and the endoplasmic reticulum was, in part, greatly dilated and contained evenly scattered fine granular material. Occasional clusters of irregular, round, or elliptical lipid-containing vacuoles, surrounded by a narrow homogeneous rim of electron-dense material, were seen in the cytoplasm. These vacuoles varied greatly in size. The increased number of ribosomes gave a granular

appearance to the cytoplasm. The mitochondria were increased in number and electron density; some were elongated while others were irregularly round or oval. Bundles of densely packed tonofibrils were usually present in the cytoplasm and practically filled the cytoplasmic extension of these cells (Fig. 46).

The most interesting finding was the presence of groups of distinctive junctional complexes of different types. Near these junctions collections of rod-like structures measuring up to 10 mμ in width similar to microvilli of the intestinal epithelium were seen, tightly packed between adjacent glial elements. In some sections it was evident that they were continuous with glial processes. Occasionally a few of these villus-like processes were seen amongst coarsely granular electron-opaque material (Fig. 47). The glial cells, situated in the outer layers of the retina, had pale vesicular cytoplasm containing a few mitochondria and small amounts of endoplasmic reticulum, which was sometimes dilated and arranged in a concentric pattern, mostly located to one side of the cell. The tonofibrils were rarely found in these cells.

(*ii*) *Outer Plexiform Layer*.—In addition to the atrophy of the dendrites some of the synaptic bodies of the receptor cells appeared abnormal and sometimes showed small, contracted, synaptic lamellae.

Retinal Macrophages

These cells invade the affected area, penetrate the cellular debris, and embrace, engulf, and finally digest the necrotic material. It was of particular interest to try to distinguish the cell types engaged in phagocytic activity in the specimens described. At the height of phagocytosis, when the cells were practically full of ingested material, this was impossible, but in the early or terminal stages two main cell groups could be identified. Some of the macrophages appeared round or oval and often had only small, short cytoplasmic expansions. The cytoplasm contained few mitochondria, relatively small amounts of rough-surfaced endoplasmic reticulum, a Golgi complex, infrequent ribosomal particles, a well-developed centrosome, and characteristic granules. These granules, measuring between 0·2 and 0·5 μ, were round or oval, homogeneous, electron-dense bodies usually surrounded by a membrane. Intracytoplasmic inclusions could often be seen similar in nature to the degenerating elements outside the cell boundary. The nuclear chromatin was dense and collected into clumps usually at the nuclear membrane (Fig. 43).

Other cells were elongated with an eccentric, occasionally bi-lobed nucleus containing a nucleolus. In the cytoplasm a number of mitochondria and numerous ribosomal particles, a Golgi complex, a moderate amount of rough-surfaced endoplasmic reticulum, and a centrosome were present. The long pseudopodia of these cells were seen surrounding the degenerating tissue elements and characteristic cytoplasmic granules were occasionally seen (Fig. 48). A number of transitional and pleomorphic macrophages were observed exhibiting an electron microscopical appearance somewhat similar to the cells described above. It is possible, therefore, that these are all phases of the same cell type.

Cells having identical characteristics of the macrophages seen in our experiments are to be found occasionally in the normal retina and adopt the shape of the space

they occupy (Fig. 49). These cells are the histiocytes (clasmatocytes, adventitial cells, wandering cells) of the normal retina which are identical with the histiocytes of other organs in the body and become active in the presence of cellular degeneration. Their origin and function will be discussed later (*cf.* Phagocytosis, p. 345).

The macrophages of the retina are apparently actively motile and can squeeze into and penetrate the extracellular spaces between adjacent cells, with intact plasma membranes, to reach the particle to be ingested. In our specimens the cytoplasmic processes of the macrophages could be seen extending towards the disintegrating material and practically surrounding it. At a later stage fusion of the opposing cytoplasmic extensions occurred, thus resulting in complete enclosure of the cellular debris and the formation of a phagocytic inclusion, limited by the invaginated cell membrane (Fig. 48). At a later stage, when the digestion of the engulfed material began, the contents of the phagocytic inclusion appeared as a vacuolated, irregularly electron-opaque, round or oval mass in which some remnants of the structure of the ingested material could be discerned. The vacuolated or clear areas in the masses may have represented unsaturated fatty acids which are reduced during osmium tetroxide fixation and dissolved in the process. As phagocytosis progressed the inclusion became a homogeneous, finely granular mass of medium electron density, containing spiral linear structures of high electron density. The membrane limiting the mass at this time was partially disrupted and, as phagocytosis continued, the inclusion became paler and broke up into smaller masses of varying size, shape, and electron density with no limiting membrane. In some cases the membrane of the phagocytic inclusion disintegrated and the debris became diffusely distributed in the cytoplasm of the macrophages.

During the course of phagocytosis the macrophages, or gitter cells, underwent considerable changes. When the entire cytoplasm of the cell was almost filled by a phagocytic inclusion, the nucleus appeared indented and pushed towards one pole of the cell close to the plasmalemma. The cells appeared elliptical in shape and their cytoplasm was confined to a narrow rim near the plasma membrane. There was an increase in the number of intracytoplasmic ribosomes giving an overall granular appearance to the cytoplasm which also contained a number of vacuoles without limiting membranes, and which were partially filled with what appeared to be lipoid material. Of especial interest was the behaviour of the characteristic cytoplasmic membrane-bound granules or dense bodies, which were situated very close to the material to be digested and appeared vacuolated where they made contact with the phagocytic inclusion. Occasionally they were seen inside the phagocytic mass. At times, however, it was difficult to distinguish between these granules and those derived from the breakdown of the phagocytic masses (Figs 44 and 45).

The large phagocytic masses finally disrupt into many smaller relatively homogeneous clumps of varying shapes and dimensions, without distinct boundaries, and which merge into the cytoplasm. The macrophages now contain only a few small, pale phagocytic lumps accompanied by what appear to be lipid vacuoles. The occurrence of macrophages near the blood vessel is too frequent to be accidental, and they usually show direct contact between their plasma membranes and the basement membrane of the vessels. In some instances long projections of these cells extend towards a vessel (Fig. 43).

Haemorrhages

A most interesting finding was the presence of filamentous material, erythrocytes, and leucocytes in the enlarged intercellular spaces of the area of haemorrhage (Fig. 50). Under high magnification the filaments occasionally showed cross-striations with a periodicity of about 150–200 Å, characteristic of fibrin (Fig. 51). It was surprising to note that the enlarged intercellular spaces were surrounded by apparently normal neural and glial elements with intact plasma membranes. The extracellular material was either closely abutting the intact membranes of the surrounding cells, or was free in the large spaces which often contained fine, widely scattered, granular material arranged in a reticular pattern. The extracellular material extended from the inner limiting membrane to the synaptic bodies of the receptor cells. In the extracellular spaces macrophages were often seen in close proximity to the erythrocytes and appeared to have a striking affinity for fibrin, which could be seen within their membranous invaginations or short cytoplasmic processes (Fig. 52). Some of these macrophages were filled with fibrin and erythrocytes which were in different stages of erythrophagocytosis (Fig. 53), while other macrophages were elongated, stretching towards the extracellular fibrin (Fig. 52). Of especial interest were the intracytoplasmic membrane-bound vacuoles, which contained heterogeneous granular material incorporating electron-opaque bands. These dense bands were sometimes situated in the intracytoplasmic membrane-bound dense bodies (Figs 53 and 54). Decreased electron density in the glial fibres and rupture of their membranes with liberation of their cytoplasmic vesicles and mitochondria were rarely encountered. These changes in the glia, however, may be due to artefacts.

Vascular Changes

Structural changes occurred in some of the vessels while many retained their normal appearance throughout the different periods of experimentation. After one hour of embolization the lumina of the affected capillaries, particularly those in the deeper layers of the retina, were distorted and narrowed. Occasionally they were almost closed, but their cellular structures were unaltered. After five hours the capillary cells showed more abundant ribosomes and appeared granular; the nuclear chromatin was denser and clumped at the nuclear membrane. The changes in the basement membrane were striking; it appeared thicker, tortuous, and smudged, with an ill-defined border (see Fig. 20). By seven days the intramural pericytes showed definite changes consisting of intracytoplasmic lipid droplets, granular and lamellar arrays of electron-opaque material, with a transverse or concentric pattern. Occasionally a glial process appeared to have penetrated the lodge of an intramural pericyte, through a defect or split in the basement membrane, and had surrounded a dense membranous and granular mass, probably a degenerate pericyte (Fig. 55).

After a longer period of experimentation (particularly at 14 to 25 days), irregular rings of basement membrane-like material were seen to be surrounded and lined by the cytoplasmic extensions of the reactive glia, recognizable by its smooth-surfaced membrane-bound vesicles and bundles of tonofibrils. Between the plasma membranes of the lining cytoplasmic extensions a fibrillar material of low electron density was occasionally seen (Fig. 56). It must be remembered that these areas

could be interpreted as tangential sections through distorted vessel walls, but they are more probably remnants of basement membrane with a reactive gliosis.

During sectioning the ballotini were sometimes encountered and they could be easily recognized by inspecting the Epon block with a binocular microscope. Thin sections of an arteriole where the ballotini had lodged showed compressed and shrunken endothelial cells (Fig. 57), the endothelial matrices of which were condensed to a homogeneously electron-dense material. Sometimes endothelial cells were completely absent and a dense membrane-like linear opacity partially covered the basement membrane, which appeared to be particularly condensed in the centre of the intracellular spaces. In a few instances the underlying muscle cells contained many lipid droplets.

An interesting finding in the perivascular glial cells was their watery cytoplasmic appearance. Some of them seemed to be empty with disrupted plasma membranes, and a sparse number of vesicles and tonofibrils were confined to the periphery of the cells. It is unlikely that the changes in the glial cells were due to artefact.

DISCUSSION

As was to be expected, micro-embolization of the retinal arterioles resulted in severe injury to the retina with extensive structural alterations. Although these changes were predominantly within the inner layers of the retina, some degeneration was found also in the outer layers, since the Müller cells ramify widely throughout these layers also. The sequence of degenerative changes in the neural, glial, and vascular systems of the retina after embolization, and their eventual phago-cytosis and replacement by glial proliferation will now be discussed in relation to the literature.

Nerve-fibre Layer and Ganglion Cells

Nerve Fibres

(a) Swelling and Mitochondrial Changes.—A review of the literature regarding electron-microscopical changes of the nerve fibres in the central and peripheral nervous system reveals that many experimental methods have been employed to produce degenerative and regenerative changes in the axons, dendrites, and peri-karyons, such as cutting or crushing the nerve fibres, subjecting them to hypoxia, toxins, or vitamin E deficiency, etc. In addition, the pathology of many naturally occurring disorders of the nervous system has been studied with the electron micro-scope, but except for two reports from our own laboratory (Ashton and Harry, 1963; Dollery, Ramalho, Paterson, Hill, Henkind, Shakib, and Ashton, 1965) we are unaware of any studies dealing with the degeneration of retinal nerve fibres. The present investigation has shown that many of the pathological changes described in the central and peripheral nervous system, whether due to disease or experiment, can be duplicated in the retina by arteriolar occlusion; indeed, as we have already pointed out, the retina is an ideal tissue in which to study these changes since it provides a perfect correlation between the pathological developments which may be observed in living and fixed tissues.

In 45 to 60 minutes after occlusion of the arterioles by ballotini the ischaemic area usually appeared white and oedematous and electron microscopy revealed greatly distended axons among axons of normal diameter. This early distention seemed to be due to swelling of the axoplasm, since it occurred even where the neurofilaments were sparse and there was no proliferation of the axonal organelles. In axons with normal diameters, however, and in axonal areas prior to the swelling there was a notable increase in the number of mitochondria and membrane-bound microvesicles; microdense bodies were also present and these structures all tended to aggregate at certain points in the nerve fibre.

The fate of axons showing simple swelling is not clear; whether they shrink again or rupture or pass into a proliferative phase, as seen in the axons at a later stage, cannot at the moment be determined. In short, we do not know whether simple axonal swelling is a precursor of swelling with proliferative changes, or whether they are separate reactions. It is to be noted, however, that, as seen by light microscopy, simple swelling occurs predominantly in the centre of the lesion, whereas swelling with proliferation develops at the periphery.

At this early stage the ganglion cells were depleted of cytoplasmic organelles at the side of the axonal hillock, suggesting that they might have migrated into the nerve fibres by means of axoplasmic flow to form the aggregations. There is, however, an alternative explanation, for the decrease in organelles in the vicinity of the axonal hillock might be due to redistribution in other regions of the perikaryon consequent upon changes in cell volume.

A few hours after embolization these changes had progressed with swelling of the axons containing the organelles, in addition to simple axonal swelling. Similar focal aggregations of axonal mitochondria were observed by Webster (1962) in an electron-microscopical study of the early stages of Wallerian degeneration after crush injury in the sciatic nerve of the guinea-pig. These accumulations of mitochondria preceded or accompanied the axon swelling and he suggested that they developed through migration or proliferation of the pre-existing axonal mitochondria. Lubínska (1964) showed that in a crushed sciatic nerve of the rabbit structural and biochemical changes occurred on both sides of the lesion. According to him this is due to a mere translocation of pre-existing enzyme-containing particles, carried within the stream of axoplasm to settle at the injured or cut ends of the axons, which would necessarily imply that the axoplasm streams in both an ascending and descending direction. Although it is true that the region of the axonal hillock becomes depleted of organelles, our other observations cast some doubt on the hypothesis of the migration of pre-existing organelles, for we have found that an axon undergoing degeneration might have several localized areas of expansion containing supernumerary organelles, but the distribution in the intervening segments was essentially normal, whereas, if mitochondria were simply accumulating focally in the axons, one might expect a depletion of these organelles in other areas of the same axon. Moreover, since the mitochondria and other organelles appeared in numbers far in excess of normal they must proliferate at some site. We could find no evidence that mitochondria were being manufactured in the perikaryon in response to injury to the axon. On the contrary, a depletion of the intracytoplasmic organelles on the side of the axon hillock of the perikaryon invariably occurred, that is, in an area where crowding of

mitochondria might be expected if the cell were a constant source of the proliferating organelles.

We interpret our findings as more in favour of a proliferation *in situ* of the pre-existing mitochondria in the degenerate regions of the axons, as suggested by Schlote (1964). As this process progresses a segmented enlargement of the axons occurs, which differs from the axoplasmic swelling seen at an earlier stage in being due to proliferation of mitochondria and other elements, but we hesitate to support the concept that these structures migrated by axonal flow. Focal proliferation of mitochondria appears to be a non-specific response to injury.

In common with other workers we have noticed that many axons appear normal in a section of the lesion. This appearance, however, may be misleading, for it is apparent that even a damaged axon may be ultrastructurally normal in some areas along its course. Thus not only different axons, but also differing parts of the same axon may show unequal resistance to injury. Likewise, in many peripheral neuropathies, the distal part of the nerve demyelinates before the more proximal parts (Lubínska, 1964), and a patchy distribution of degeneration may result in axonal injury from organo-phosphorous poisoning (Cavanagh, 1954; Majno and Karnovsky, 1961).

(b) Membrane-bound Microvesicles.—The role of the microvesicles which appear in the earlier specimens is unknown. They may be similar to the vesicles found in synaptic endings (Estable, Acosta-Ferreira, and Sotelo, 1957), which in a presynaptic site are believed to play a role in transmission of nerve impulses, but the same type of vesicle is also found in the post-synaptic sites (Missotten, 1965). In any event it would seem that this hypothesis of transmission cannot be extended to degenerating nerve fibres. It has been suggested that vesicles may have a metabolic or plastic function and may be precursors of neurofilaments (De Robertis and Sotelo, 1952; Estable and others, 1957) and it is interesting to recall that in our 24-hour specimen the number of microvesicles had substantially decreased, whereas the neurofilaments and dense bodies had increased. The literature on this subject is not, however, entirely conclusive. De Robertis and Sotelo (1952) studied growing nerve fibres in tissue culture by electron microscopy and described vesicles at first aligned in rows and later coalescing to form "neurotubes", but transitional forms between vesicles and neurofilaments were not found. Neither Terry and Peña (1965), who produced neurofilamentary hypertrophy in rabbits by alum phosphate, nor Guillery (1965), who obtained a similar hypertrophy in the severed optic nerve of monkeys, demonstrated microvesicles prior to the formation of neurofilaments. The observations of both these groups of investigators, however, were made some days after experimentation so that the vesicular stage may have been missed.

Our experiments suggest that microvesicles and tubules are formed, like mitochondria, in response to injury, possibly as a proliferation of endoplasmic reticulum (Lampert, Blumberg, and Pentschew, 1964), and the observation of Luse and Smith (1964), that numerous microvesicles may be found in the degenerating neurites of senile plaques of the brain supports this view. As a tentative conclusion we would suggest that the molecular components of these microvesicles may possibly be re-orientated into neurofilaments and mitochondria, or, alternatively, may simply form microdense bodies.

(c) Microvesicular Bodies.—These were occasionally seen in our specimens and were usually associated with mitochondria, of which they appeared to be a breakdown product. Similar structures have been described in degenerating nerve fibres in vitamin E deficiency (Lampert and Pentschew, 1964), and in growing cones of sectioned nerve fibres (Estable and others, 1957), but they differ from the membrano-vesicular bodies described by Gonatas, Terry, Winkler, Korey, Gomez, and Stein (1963) in juvenile lipoidosis, for these bodies were larger and showed an internal laminated structure.

(d) Dense Bodies.—These structures became more conspicuous in the three-day specimens and were of different types. Some were certainly condensed mitochondria and membrane-bound vesicles, while the derivation of others was less apparent. The possibility that mitochondria and tubular profiles are derived from them (Lampert and others, 1964) can be ruled out, since dense bodies were rarely present in the earliest stages (five-hour specimen) when mitochondria and membrane-bound vesicles and tubules were most numerous. As suggested by D'Agostino (1964), a few of these dense bodies may be acid phosphatase-rich granules, termed lysosomes, which are known to increase in neural cytoplasm after injury, as in sectioned axons. Others may be lipofucsin or lipofucsin bodies. Indeed, Gonatas and others (1963) believe that lysosome-like bodies (lysosome-*like* because acid phosphatase activity was not actually demonstrated), membrano-vesicular bodies, and lipofucsin are morphological units representing a chain of chemical and associated structural transformations which end in the lipofucsin body. Our observations support this concept of a morphological progression of the products of neuronal degradation, and, in the final stages, all these structures appear to conglomerate into masses of lipid material.

(e) Neurofilaments.—These first became conspicuous after 72 hours of embolization; then the swollen axons became filled with densely packed neurofilaments ("torpedoes") which displaced the other organelles towards the axolemma. As already pointed out, our findings corroborate the theory of Gray and Hamlyn (1962) and of Guillery (1965) that membrane-bound microvesicles are formed during the early stages of the degeneration and are later replaced by neurofilaments. It is clear that the filaments must either invade the terminals from the axons or be synthesized *in situ* in the degenerating axon terminal (Guillery, 1965); we are in favour of the latter alternative, since the number of neurofilaments, as seen in longitudinal sections, was not increased in regions proximal to the torpedoes. There is a possibility, however, that the protein precursors of the filaments may be synthesized in the ganglion cells and the axons and only precipitated in the torpedoes in filamentous form. Evidence for such plasticity of fibre proteins has been provided in studies of the axoplasm of the squid (Davison and Taylor, 1960; Schmitt, 1961), wherein it was shown that neurofilaments consist essentially of chains of globular protein units constrained into a helix by electrostatic forces, and may disassociate and reassociate into fibre form by *p*H changes or the action of salt.

Hypertrophy of neurofilaments in degenerating nerve fibres has been described by many authors (Gray and Hamlyn, 1962; Lampert and others, 1964), and discussed in detail by Guillery (1965). That they are evidence of regenerative activity was

originally suggested by Cajal (1928), and it is now known that both ends of a cut nerve may have the same regenerative capacity (as also of mitochondrial proliferation). Indeed, we have seen accumulations of organelles and neurofilaments in several foci along a single axon, and regard it as a non-specific response to injury, which in our case was ischaemic anoxia. It will be recalled that neurofilamentous tangles are a feature of Alzheimer's disease; these, and the formation of filaments in severed and crushed nerves, suggest that a faulty metabolic function may cause the precipitation of soluble protein in a filamentous form, and it is interesting to note that Terry and Peña (1965) have produced neurofilaments experimentally in rabbits by injections of alum phosphate.

The significance of this neurofibrillar hypertrophy is not clear, nor is this surprising, since the normal function of neurofilaments is by no means understood. The obvious possibility that they are concerned with the conduction of nerve impulses is no longer tenable (Parker, 1929) and the suggestions that they serve a supporting (Parker, 1929) or a memory function (Schmitt, 1961) must also be rejected. At the present time it would seem most likely that the neurofibrillar hypertrophy is an attempt to re-establish the proper connexions of the injured nerve (Guillery, 1965).

(f) Membranous Whorls.—These structures first appeared 24 hours after embolization and were conspicuous after an interval of 72 hours. Similar ribosome-free whorls have been seen in a number of normal cells—in neurons of goldfish (Robertson, Bodenheimer, and Stage, 1963) in sympathetic ganglion cells of rats (Palay and Palade, 1955); in the transitional epithelium of the urinary bladder (Walker, 1960), and in the parathyroid gland (Trier, 1958). We have, however, never seen them in the normal axons or ganglion cells of the specimens we have studied. The whorls have also been described in pathologically altered cells—in liver cells injured by ethionine (Steiner, Miyai, and Phillips, 1964), in Ehrlich's ascitic tumours, cells infected with Newcastle-disease virus (Adams and Prince, 1959) and in argyrophil cells of gastric mucosa after fasting and water deprivation (Helander, 1961). Post-mortem changes in the cells of the stomach and the kidney of bats produced whorls resembling myelin figures which were, however, considered to be the plasma membrane of the microvilli (Ito, 1962). Other types of layered loops of membranes in dystrophic axoplasm after vitamin E deficiency were described by Lampert and Pentschew (1964), and Lampert and others (1964), but they were unable to demonstrate any derivation from the axolemma, the endoplasmic reticulum or the neurofilaments. Round or irregularly oval structures, larger than mitochondria, bound by a single membrane and containing aggregates of linear densities were described by Gonatas, Levine, and Shoulson (1964) in juvenile lipoidosis, and designated as "membranovesicular bodies". These authors point out the existence of similar structures in the phagocytic glia in Tay-Sach's disease. They also describe cytoplasmic organelles which somewhat resemble membranous cytoplasmic bodies of Tay-Sach's disease, which consist of closely packed electron-dense membranes, frequently arranged concentrically, and containing centrally a homogeneous or finely granular zone. We have been able to produce very similar inclusion membranous whorls in the retinae of kittens by irrigating the retina *in situ* for 10 minutes with 2·7 per cent. sodium chloride (Shakib, 1966a).

Terry and Weiss (1963) have divided cytoplasmic membranous whorls into four groups according to type and possible origin: (*a*) unusual figures of Golgi apparatus; (*b*) uncommon whorls of ergastoplasm; (*c*) multilaminated mitochondria; and (*d*) membranous cytosomes unrelated to the cellular organelles. According to them the membranous cytoplasmic bodies in Tay Sach's disease belong to the last group.

As can be seen from the literature and our own findings the different types of membranous structures are associated with degenerating cells, and it seems clear that the presence of membranous arrays in the axons is an indication of pathological alteration in the cytoplasm. They may represent accumulations of lipoprotein molecules, for very similar laminated myelin figures have been produced in model systems using purified lipids and proteins (Stoeckenius, 1962; Samuels, Gonatas, and Weiss, 1965).

(g) End Stage of Axonal Changes.—In the final stages the axonal elements condensed so that individual components were barely recognizable and the contracted axon appeared as a clump of electron-dense, coarsely granular material, which became engulfed by macrophages. Very occasionally, where degenerating axons were not ingested by macrophages, they remained in the nerve fibre layer as hyaline bodies.

Ganglion Cells

The electron-microscopical appearances of degenerative changes in ganglion cells elsewhere have been studied by a number of workers following axonal section and toxic injuries, and we shall attempt to correlate our findings with theirs. A peripheral shift of the nucleus in the early stages of chromatolysis has been remarked upon (Hartmann, 1954; Causey and Hoffman, 1955; Smith, 1961; Evans and Gray, 1961), but since the nuclei of retinal ganglion cells are generally eccentric this could not be appreciated in our specimens. The early depletion of organelles in the cytoplasm on the side of the axonal hillock, to which we have already referred in discussing axonal changes, does not appear to have been recorded previously at this site. Reduction of ribosomes in the injured cell has been noted by others and may be due to the liberation of ribonucleic acid (RNA) in a soluble form (Gray, 1964). Using a microchemical method Hydén (1960) found that in the early stages of chromatolysis the total RNA remained constant, but the clumps broke down into fine particles. It is possible that the soluble RNA is carried by axoplasmic flow into the axonal torpedoes to play some role in the organization of the structures which proliferate at this site.

The mitochondrial swelling and dilatation of the endoplasmic cisternae, which we found as early as 1 hour after embolization and most markedly at 24 hours when they became vesicular, has also been seen in ganglion cells injured by toxic substances, by D'Agostino (1964), using plasmocide in rats, and by Anderson and Van Breemen (1958), using malononitrile in frogs, and in chromatolysis following section of the nerve, by Barton and Causey (1958), and even in the degenerating axon itself (Vial, 1958; Ohmi, 1961; Lee, 1963).

The next stage in ganglion-cell degeneration in our specimens was conglomeration of the hyaloplasm into granular masses containing the remnants of ergastoplasm,

dense bodies, and mitochondria; the nuclear chromatin clumped, the nuclear membrane broke down, and the products of this disintegration were phagocytosed. We know therefore that most of the injured ganglion cells did not recover, but at the 3- or 7-day stage we have encountered cells with a relatively normal appearance lying adjacent to degenerating cells. It is possible that these cells had survived the injury, for it has been shown by Causey and Hoffman (1955), Smith (1961), and Gray (1964) that recovery and reconstitution of the perikaryon (chromatosynthesis) may follow section of the nerve fibres. It would appear that the ability to recover probably depends not only upon the nature and site of the injury but also upon the particular cells involved.

Fluorescein angiography indicated that there was a back flow of blood through capillary anastomoses into the capillaries of the occluded area, and this may have been adequate for the survival or recovery of some cells. As in the case of nerve fibres we have no absolute answer as to why one cell undergoes degenerative changes and complete destruction, while an adjacent cell may appear absolutely normal.

In Wallerian degeneration of crushed or severed nerve fibres several authors have tried to relate the varying vulnerability of the axons to the differences in their diameter. Ohmi (1961) found that Wallerian degeneration appeared later in larger myelinated fibres than smaller ones, whereas Taxi (1959) and Lee (1963) found larger myelinated nerves more vulnerable. These conflicting results suggest that the width of the nerve fibre is unlikely to play any very definite role in determining vulnerability.

If the injury is severe all the ganglion cells undergo degeneration, and complete atrophy ensues, but axonal swelling and organelle proliferation, being reactive processes, occur only while life still exists. For instance, total ischaemia of the retina and choroid has been induced experimentally in our laboratory by raising the intra-ocular tension and we have never encountered axonal changes after this generalized injury. They have been found only in focal anoxic or hypoxic lesions. Thus the formation of axonal torpedoes in the area of micro-infarction could be the result of circulatory damage to the axons of uninjured ganglion cells outside the ischaemic area, and to the axons of surviving ganglion cells within this area. The sequence of events seems to be that the nerve fibres are affected first, since they show profound swelling after only one hour of arteriolar occlusion, when ganglion cells appear relatively normal. If these axons are from irreparably injured ganglion cells in the area of occlusion, then they undergo complete degeneration, but if they are from unaffected or surviving ganglion cells then they may develop torpedo formations.

Inner Plexiform Layer

This layer of the inner retina, we have found, is most sensitive to anoxia and readily undergoes post-mortem change. Minimal fixation artefact can be demonstrated in this area. In the presence of degenerative changes it is extremely difficult to differentiate between dendrites and axons in this region, and this will not, therefore, be attempted. The degenerative changes observed in our experiments were similar to those described by De Robertis (1956) in the acoustic ganglion of the guinea-pig after nerve section.

One hour after occlusion there was a definite swelling of the nerve endings with decrease in electron density of their matrix. After five hours the swelling was extensive and most of the synaptic vesicles had disappeared and were replaced by a finely granular material. Some of the mitochondria were swollen and a number of nerve endings seemed to be condensing to electron-dense material.

At 24 hours the few synaptic vesicles remaining had clumped, and together with swollen mitochondria, dense bodies resembling condensed mitochondria, and fragmented ergastoplasm, formed conglomerations in the swollen vacuolated nerve endings.

After 72 hours almost all the degenerating endings were aggregated into dense, irregular masses in which the structural components were difficult to distinguish. After removal of these necrotic masses by macrophages, atrophy of the layer followed. Generally the nerve endings became swollen in the acute phase of ischaemic anoxia, but some appeared to shrink without initial swelling.

In no instance did we encounter a dense plexus of neurofilaments in the degenerating perisynaptic fibres; this was in accord with the observations of De Robertis (1956) on the degenerating acoustic ganglion, but in contrast to those of Gray and Hamlyn (1962) and of Guillery (1965). This difference in degenerative behaviour is puzzling since there is little or no difference between the morphological appearances of these synaptic endings. None of the proliferative changes found in the axons of the ganglion cells was seen in the axons of the bipolar cells, although they were subjected to identical experimental conditions. This may be due to the fact that the whole cell and its branches are involved in the infarcted area, and the injury being more complete results in necrosis rather than a proliferative response. We have produced this type of necrotic change in retinal and choroidal ischaemia induced by raising the intra-ocular pressure (Shakib, 1966b).

The electron-microscopical appearances of the degenerative changes which develop in the optic tectum of the chicken after section of the contralateral optic nerve were studied by Gray and Hamlyn (1962). In the early stages optic axon terminals were plentiful and packed with neurofilaments, but eventually these completely disappeared and since no macrophagic activity was found it was presumed that all necrotic material was removed by extracellular enzymes. Walberg (1963) observed that multivesicular inclusion bodies appeared in great numbers adjacent to the degenerating buttons, and believed that the latter were partly removed by pinocytic activity of normal dendrites. Our experiments, however, showed active macrophages removing the degenerated nerve endings in the 3-day and older specimens.

Inner Nuclear Layer

Here the pattern of degeneration after infarction is similar to that seen in the neurons of the cerebral cortex after death (Karlsson and Schultz 1966), following acute hypoxia and histotoxic hypoxidosis produced with potassium cyanide (Hager, 1963). The degenerative sequence in the retina did not appear to be specific to the neurons, for, at the later stages, no differentiation could be made between the neural and glial elements. Some of the cells were greatly altered after only one hour of arteriolar occlusion and the less damaged cells showed swelling of both the mito-

chondria and the cisternae of the endoplasmic reticulum with reduction in electron density of the cytoplasm. Nuclear alterations were clearly detectable in the very earliest specimens. Similar changes have been observed in other tissues subjected to hypoxia; in liver cells (Mölbert and Guerritore, 1957) and in myocardial ischaemia and early infarction (Caulfield and Klionsky, 1959). Müller cells appeared to be more resistant to anoxia than the bipolar cells, as already suggested by light microscopy in the case of these and other glial cells (Adams, 1958; Wolter, 1959a).

In later stages the endoplasmic reticulum formed circular and oval sacs of varying dimensions and their associated ribosomes were lost. This was followed by marked swelling of the entire cell with rupture of the cell membrane and disintegration of the mitochondria and Golgi system; intracytoplasmic dense bodies were present. Finally, the necrotic cells appeared as granular osmiophilic masses in which clumps of nucleoplasm could be distinguished. These changes are purely autolytic and have been attributed to the release of hydrolytic enzymes from injured lysosomes (Beaufay and de Duve, 1959; de Duve and Beaufay, 1959), but we have been unable to identify such structures in the inner layer of the pig's retina. On the other hand, lysosome-like bodies have been reported in nerve cells by Palay and Palade (1955), and by Hudson and Hartmann (1961), and have been shown to form in liver cells of rats subjected to hypoxia (Confer and Stenger, 1964), and may accumulate in cells in the process of ageing (de Duve, 1963). According to Hager (1963) some of the dense bodies found in the neurons of the cerebral cortex after hypoxia contain hydrolytic enzymes and could be responsible for degeneration and lysis of the cells; a concept supported by the work of Becker (1963). Contrary evidence, however, has been provided by Zeman (1963) who was unable to find any variation in hydrolytic enzyme activity in lytic necrobiosis of nerve cells damaged by hypoxaemia. Our own experience in the case of the retina is in accord with this, for, although dense bodies were found in the degenerating cells in the inner nuclear layer, they formed too late and were too few to be of significance in the autolytic process.

Outer Plexiform Layer

The alterations and sequence of degenerative changes in the dendritic processes of this layer were similar to those of the inner plexiform layer. The configurations of the synaptic bodies of the visual cells and their synaptic lamellae were altered, and the number of synaptic vesicles appeared to be reduced. Transneural alterations in the uninjured synaptic endings, resulting from injury to their synaptic counterpart, have been reported by Gray and Hamlyn (1962), who described loss of postsynaptic thickening due to disappearance of presynaptic buttons after sectioning of the presynaptic nerve fibres. To reach a definite conclusion on the transneural alterations in the synapses of the visual cells, further investigation over a longer period and with more detailed study is necessary.

Phagocytosis

Since the early work of Del Rio-Hortega (1921) it has been recognized that the phagocytic element, or "microglia" of the central nervous system, is of mesoblastic origin and of the same nature as histiocytes or macrophages in general. It is known

that they reach the brain at the time of its vascularization during embryonic life and enter a resting phase within the tissue. This is true also of the retina which contains phagocytes only after vascularization (Wells and Carmichael, 1930; Jablonski and Meyer, 1938), and it has been demonstrated that macrophages in fact accompany the retinal vascular ingrowth (Ashton, 1966) and are identical with those described elsewhere in the body (Jiménez de Asúa, 1927) which we have confirmed with the electron microscope.

In pathological conditions, however, it is not easy to determine to what extent the macrophage reaction is attributable to local proliferation of resting cells or to invasion from the blood stream, and many electron microscopical investigations of the central nervous system (Schultz and Pease, 1959; Bunge, Bunge, and Ris, 1960; Gonatas and others, 1964), and of other tissues (Karrer, 1960) have failed to ascertain the origin of macrophages. In the case of the brain, however, it is generally accepted that at least some of the macrophages arise locally (Schultz and Pease, 1959; Bunge and others, 1960; Konigsmark and Sidman, 1963; Gonatas and others, 1964), but a haematogenous component has been disputed by Hain (1963) who, after destroying the circulatory monocytes by irradiation, concluded that the cerebral macrophages arose locally. In allergic encephalitis, however, haematogenous monocytes are at least one type of macrophage and have been demonstrated passing through the capillary wall (Bubis and Luse, 1964; Lampert and Carpenter, 1965).

It would seem that a breakdown in the integrity of the capillary endothelial membrane would be essential to permit the passage of macrophages through the vessel wall. If this is so, and believing that the blood–retinal-barrier to fluorescein depends upon an intact endothelium (Ashton, 1965; Cunha-Vaz, Shakib, and Ashton, 1966), the fact that by angiographs we could find no fluorescein leakage in the ischaemic retinal areas suggests to us that the phagocytic response was entirely of local origin, without a contribution from the blood stream. This would not, however, exclude the vessels as a potential source of macrophages when the blood–retinal-barrier is destroyed, as seen in the comparable case of breakdown in the blood–brain-barrier in allergic encephalitis (Bubis and Luse, 1964; Lampert and Carpenter, 1965), and in stab wounds (Schultz and Pease, 1959).

The macroglia, as well as the microglia, is involved in phagocytosis, as shown both by light microscopy (Ferraro and Davidoff, 1928; Cramer and Alpers, 1932) and by electron microscopy in the case of astrocytes (Luse, 1958; Terry and Weiss, 1963), and of oligodendrocytes (Gonatas and others, 1964). Our experiments demonstrate that retinal astrocytes have some role in phagocytic activity, for many gitter cells still show the characteristics of astrocytes, but it should be emphasized that most of the macrophagic reaction in the ischaemic retinae consisted of histiocytes.

The fate of macrophages, like their origin, is a controversial subject. In no instance were we able to find a macrophage entering a blood vessel, although many were found lying against vessels, and this is in accord with observations on spinal cord injury (Bunge and others, 1960). It may be countered that the probability of finding a cell actually migrating through the vessel wall is remote, but many authors have made such observations without apparent difficulty (Bunge and others, 1960; Bubis and Luse, 1964; Lampert and Carpenter, 1965).

In studies by light microscopy Adams (1958) observed that phagocytes eventually

come to lie close to vessels and become smaller and flatter as they apply themselves to the walls. Our studies show this also, but whether they then discharge their phagocytosed material into the vascular lumen and resume their life as inactive histiocytes or actually migrate into the vessels, cannot as yet be determined.

Glial Cells

Histologists using conventional techniques, especially silver impregnation, have described the different types of glia to be found in the retina (Cajal, 1911; Polyak, 1941; Wolter, 1955, 1959b). Wolter has described four different types: astroglia, radial fibres of Müller, perivascular glia, and oligodendroglia. The few electron-microscopical studies of the retina (Fine and Zimmerman, 1962; Hogan and Feeney, 1963) have not yet established the presence of oligodendrocytes similar to those seen in the central nervous system, which have a denser cytoplasm and nuclear chromatin than the astrocytes (Hartmann, 1958; Palay, 1958; Schultz, 1964).

In our laboratory we have studied several species of vertebrate retina (human, monkey, pig, rat, cat, dog, and rabbit) and found astrocytes in all specimens, but oligodendroglia were found only in the rabbit, forming the myelin sheath of the myelinated nerve fibres. Astrocytes are more frequently found near the disc or around the vessels, usually in the inner layers of the retina, particularly in the nerve-fibre layer close to the internal limiting membrane. They may, together with the inner cytoplasmic extensions of the Müller cells, form the inner layer of the internal limiting membrane, and may also be seen surrounding the capillaries or the nerve fibres. These cells have a relatively pale cytoplasm containing smooth-surfaced membrane-bound vesicles, a few mitochondria, a small amount of rough-surfaced endoplasmic reticulum, infrequent ribosomes, a Golgi system, and bundles of tonofibrils. In the cell body the tonofibrils are orientated towards the cytoplasmic extensions where they are particularly evident. The nucleus, which is oval or round, occasionally lies under the internal limiting membrane; the nuclear chromatin is fairly evenly distributed throughout and contains a nucleolus. These cells may be called the "astrocytes of the retina" and are easily distinguishable from the other cells by their location and cytoplasmic appearance.

The present study has shown that an astrocytic reaction—astrocytosis and astrogliosis—similar to that in the brain (Adams, 1958) can occur in the ischaemic retina. The astrocytes were more resistant to ischaemia than the neurons and Müller cells, and soon after micro-embolization the surviving astrocytes, or those at the border of the lesion, showed increased numbers of ribosomes, mitochondria, and fine membrane-bound vesicles and tubules (some studded with ribosomes). Osmiophilic droplets, similar to those seen by Schlaepfer and Hager (1964) in proliferating fibroblasts, histiocytes, and infiltrating leucocytes appeared. At a later stage the reactive astrocytes showed bundles of densely packed tonofibrils and dilated cisternae of endoplasmic reticulum containing moderately dense material. The abundant cytoplasm and enlarged nucleus appear dense at this time.

It is interesting that at no stage in our experiments did the retina show enlargement of the intercellular or perivascular spaces, in contrast to the changes in rat brains after puncture wounds (Schultz and Pease, 1959) and in the spinal cord of barbotaged

cats (Bunge and others, 1960), wherein perivascular spaces were found. While the macrophages were active in removing necrotic cells the hypertrophic and proliferating astrocytes were replacing them, producing a remarkable pattern in the inner layer of the retina where the astrocytic processes ran parallel with the remaining nerve fibres.

Johnson, McNabb, and Rossiter (1950) have suggested that Schwann cell proliferation in Wallerian degeneration may be induced by either a chemical mediator resulting from the products of axonal degeneration (a point previously made by Abercrombie and Johnson, 1946), or a physical factor due to the space left after lysis and removal of the necrotic material. Similar factors probably operate also in retinal ischaemia, the astrocyte activity being stimulated by a chemical mediator, and their proliferation following when space is made available by phagocytosis of debris. This proliferation becomes evident without any visible mitosis and may result from many different injuries as described by light microscopists (Adams, 1958; Hogan and Zimmerman, 1962). In the final stages the Müller cells and other cellular structures in the inner nuclear layer could not be differentiated, as all the cells showed identical degenerative changes. Their hyaloplasm became condensed to a homogeneous mass of medium electron density and they further underwent the same alterations as the bipolar cells. To what extent the Müller cells took part in the terminal gliosis could not be determined.

One of the most interesting findings in our study was the aggregation of rods or villus-like structures, some of which could be seen originating from the reactive glia in the plane of section. These elements were generally arranged in parallel groups and were found at the intersection of glial cells, and differed from those described by Robertson and Vogel (1962) in oligodendroglioma in that their hyaloplasm was denser and more homogeneous.

Retinal Haemorrhages

Cotton-wool spots in the human retina are not uncommonly associated with small haemorrhages and varicose dilated capillaries (Ashton, 1959; Dollery, Hodge, and Engel, 1962; Ballantyne and Michaelson, 1962). In our experimental lesions, however, this was unusual and the occasional haemorrhages seen arose from arterioles leaking fluorescein, and were due to injury by the glass microspheres as shown electron microscopically. The presence of leucocytes, erythrocytes, fibrin, and finely reticulated material resembling plasma in large extracellular spaces, shows that such potential spaces must exist in the normal retina, but whether or not actual extracellular spaces are normally present is a controversial problem. According to Sjöstrand (1960, 1962), the peripheral layer of adjacent cell membranes, in both the brain and retina are either fused together or are separated by a space measuring only a few tenths of an Å unit containing "cement substance". In his view, which Cogan (1962) accepted, this prevents free diffusion. More recently, however, an appreciable extracellular space has been demonstrated in the brain by electron microscopy (Van Harreveld, Crowell, and Malhotra, 1965) which in extent, corresponds very closely with that estimated by inulin (Davson and Spaziani, 1959; Rall, Oppelt, and Patlak, 1962). Enlargement of the extracellular space in pathological conditions has previously been demonstrated in the brain (Lampert and Carpenter, 1965; Bubis and Luse, 1964) and in the retina (Ashton, 1965), and it is our present belief that a true

extracellular space, of dimensions yet to be determined, probably exists in the normal retina.

The mechanism of the disposal of haemorrhage in the retina is not completely understood. Ballantyne and Michaelson (1962) believe that small retinal haemorrhages lyze and are carried away by extravascular tissue fluids and so pass into the perivascular lymph circulation. It is not clear, however, what these authors mean by extravascular tissue fluids in the retina and there is no lymphatic circulation in this tissue. Our impression is that blood disposal within the retina is mainly by phagocytosis, which would explain the slow absorption of retinal haemorrhages (Hogan and Zimmerman, 1962).

Blood Vessels

It is apparent from our study that the cells composing the blood vessels are the most resistant to ischaemia. Reinecke, Kuwabara, Cogan, and Weis (1962) studied the effect of experimental ischaemia on the retinal vessels of cats, and in digest preparations found persistence of intramural pericytes despite almost total loss of endothelial cells. Using this technique, and also the method of embolization for inducing ischaemia, we have found with the electron microscope that the intramural pericytes and endothelial cells show very little difference in their vulnerability. In some areas the endothelial cells may be less damaged and in others the intramural pericytes survive, but overall we gained the impression that the latter cells are, if anything, more susceptible to anoxia. Oedema of the endothelial cells, but not of the intramural pericytes, was reported by Hills (1964) in anoxic ischaemia of rat brains, but we consider that some of her electron micrographs do in fact show changes in the intramural pericytes.

In the retina, soon after infarction, both intramural pericytes and endothelial cells contain an increased number of ribosomes, together with homogeneous osmiophilic lipid droplets with irregular borders, as already described in reactive astrocytes, Schwann cells, proliferating fibroblasts, and histiocytes.

At an early stage the lumina of the capillaries in the ischaemic area were narrowed and usually contained no red cells. In patent capillaries the endothelium appeared normal, but in narrowed vessels it was thickened and concertina-ed; no endothelial swelling was seen. It was not possible to decide to what extent capillary closure might have been due to increased tissue tension from swelling of the perivascular tissue as postulated by Ashton (1959), or to collapse of the capillaries from obstruction of the circulation and loss of endothelial tone. An interesting finding was the smudging and thickening of the basement membrane of the capillaries in the ischaemic area, which was reminiscent of the thickening seen in diabetes in the retina (Yamashita and Rosen, 1962; Bloodworth and Molitor, 1965), and in the renal glomeruli (Bergstrand and Bucht, 1959; Rees, Camerini-Davalos, Caulfield, Lozano-Castaneda, Cervantes-Amezcus, Taton, Pometta, Krauthammer, and Marble, 1964; Macdonald and Ireland, 1964), and also in hypertension in man (McGee and Ashworth, 1963) and animals (Wiener, Spiro, and Lattes, 1965). The entrance of glia through a defect in the basement membrane to surround what appeared to be a degenerating intramural pericyte was a particularly striking finding, and although we were unable to follow closely the degenerative sequence of the intramural pericyte it seems clear that glial cells participate in their removal. Endothelial

cells may also disappear and only the basement membrane persists and constitutes the intervascular strands or bridges of light microscopy. The degree of ischaemia produced by ballotini occlusion was not apparently adequate to produce endothelial degeneration and necrosis. In some of our experiments the endothelial cells appeared normal in the ischaemic area although the perivascular glia had undergone degenerative changes. No fluorescein leakage was observed in these areas, except occasionally at the site of the occluding ballotini where electron microscopy showed damage or loss of the endothelial cells, thus further supporting the concept that the endothelium is the site of the blood–retinal-barrier, at least as regards fluorescein.

Cotton-wool Spots and Cytoid Bodies

As we have already indicated, there has existed for over a century considerable controversy as to the nature of cotton-wool spots and the cytoid bodies they contain; this subject has recently been reviewed (Ashton and Harry, 1963). It is now widely believed that the cotton-wool spot is a focal ischaemic lesion of the inner layers of the retina, and it has been shown electron microscopically that the cytoid body is formed by the terminal bulbous swelling of an axon, while the pseudonucleus was thought to be due to aggregated elements within the swollen axoplasm (Ashton and Harry, 1963). In order to study this problem fresh material is essential; it was in fact for this special purpose that Ashton first entertained the idea of using embolization with glass microspheres. In what way these experimental cotton-wool spots throw light upon the structure of the naturally occurring lesions will now be considered.

From this electron-microscopical study it would seem that the white fluffy appearance of the cotton-wool spot is primarily due to intracellular swelling in the structures of the inner retinal layers together with proliferation of organelles in the axons. Simple intracellular swelling, as seen 30–40 minutes after embolization, does not apparently provide the typical picture of a cotton-wool spot in the fundus, but only a greyish-white discoloration. After 24 hours, when intracytoplasmic proliferation occurs in addition to the swelling, the typical appearances develop. The cotton-wool spot therefore represents a focal reaction of injured axons of still living cells in the inner retina. In hypertensive retinopathy focal exudation occurs in addition to swelling, as shown by fluorescein angiograms, but this evidence of endothelial damage would seem to be an additional factor rather than a primary one, for it is known that exudation alone in the retina does not necessarily produce cotton-wool spots. So closely does the experimental lesion resemble the pathological lesion in its structural changes that a common pathogenesis is a probability and, if so, this can only be focal ischaemia from total or partial arterial occlusion. Occluded arterioles, however, have as yet been demonstrated only in hypertensive retinopathy, and may be assumed to be the cause of cotton-wool spots in leukaemia, fat emboli, or sickle-cell anaemia. They have not been found in the cotton-wool spots of pernicious anaemia or after gastric haemorrhage (Ashton, Pears, and Pickering, 1961), and it may be that functional closure of the arterioles from spasm or collapse could be important in the genesis of these lesions. Further work on this aspect of the problem is required.

As regards the cytoid bodies themselves it is interesting to recall that in the past these have been variously described as degenerate ganglion cells, swollen varicose nerve fibres, necrotic mononuclear wandering cells, macrophages, degenerate glial

cells, exudate, or fibrin in precipitated ground substance, for almost all of these structures were seen in our electron micrographs. If, however, recent evidence is accepted that at least one form of cytoid body is represented by the club-shaped ends of swollen nerve fibres as seen by silver staining (Wolter, 1957; 1959b) and microdissection (Ashton and Harry, 1963), we may turn for a moment to consider the problem of the formation of this type of cytoid body and the nature of its pseudonucleus.

As we have shown, ischaemia of only a few minutes' duration results in simple swelling of the axons and this occurs focally, giving rise to nodular or varicose enlargements, as reported many years ago by Müller (1858). After a few hours some expanded axons appear filled with mitochondria, neurofilaments, dense bodies, and inclusion membranous whorls, and at this stage there may be no break or other interruption in the nerve fibres. The changes which culminate in this form of cytoid body do not therefore begin with a break as postulated by Wolter (1959b), on the basis of the findings of Cajal, but are well advanced in intact fibres. That a break eventually occurs, however, seems undeniable and the distal stump attached to the ganglion cell forms a bulb-like swelling which may develop into a cytoid body, while the proximal fibre atrophies.

We are now confident that the pseudonucleus of the cytoid body is not formed by fibrin as suggested by Christensen (1958), for in our specimens the fibrin in the intercellular spaces was never seen to penetrate the swollen axon walls. Moreover, the axonal torpedoes and accompanying glia were closely packed together without any extracellular substance intervening, so that Wolter's (1959a) suggestion that the pseudonucleus of the cytoid body is formed by the swollen axon itself while the pseudocytoplasm is some unknown substance around it, is disproved by this evidence. It has already been pointed out that artefact was probably responsible for Wolter's interpretation (Ashton and Harry, 1963).

The pseudonucleus, therefore, can be formed only by the proliferating and degenerating structures of the bulbous axoplasm, and may therefore consist predominantly or only partly of neurofilaments, mitochondria, dense bodies, or membranous whorls which become clumped into a relatively homogeneous mass some weeks after ischaemia. It is not to be expected, of course, that electron-microscopical appearances of osmic acid fixed material would exactly duplicate those of formalin-fixed material for they provide different evidence, but this interpretation of the nature of the pseudonucleus would seem to explain rather satisfactorily the varying staining reactions reported by light microscopists. (We are at present investigating the influence of delay in fixation and of formalin fixation on the cytoid body produced experimentally by embolization.)

If this conglomeration of proliferating and degenerating axonal structures does in fact represent the pseudonucleus of the cytoid body, one must consider what histological appearances might be presented when it becomes engulfed in macrophages or astroglia. It would seem possible that a typical cytoid body might also result; so that we are left with the conclusion that the pseudocytoplasm of the cytoid body may consist either of a swollen axon, or of the cytoplasm of astroglial cells or macrophages, and this would seem to bring together many of the divergent views about the nature of the cytoid body.

Acknowledgements

It is a pleasure to acknowledge with gratitude the expert technical assistance of Mr. G. E. Knight, Mr. D. Wood, Miss E. Robins, and Mrs. P. Rawlings. We are indebted to Miss E. FitzGerald and Mrs. H. Mehra for secretarial help. We are particularly grateful to the English Glass Co. for their kind co-operation.

This work was supported by a grant from the Medical Research Council and the Wellcome Trust.

REFERENCES

ABERCROMBIE, M., and JOHNSON, M. L. (1946). *J. Anat. (Lond.)*, **80**, 37.
ADAMS, R. D. (1958). In "Biology of Neuroglia" (ed. W. F. WINDLE) p. 245. Thomas, Springfield, Ill.
ADAMS, W. R., and PRINCE, A. M. (1959). *Ann. N.Y. Acad. Sci.*, **81**, 89.
ANDERSON, E., and VAN BREEMEN, V. L. (1958). *J. biophys. biochem. Cytol.*, **4**, 83
ASHTON, N. (1959). *Lancet*, **2**, 625.
——— (1965). *Trans. ophthal. Soc. U.K.*, **85**, 199.
——— (1966). *Amer. J. Ophthal.* In the press.
——— and HARRY, J. (1963). *Ibid.*, **83**, 91.
———, PEARS, M. A., and PICKERING, G. W. (1961). *Brit. J. Ophthal.*, **45**, 385.
BALLANTYNE, A. J., and MICHAELSON, I. C. (1962). "Textbook of the Fundus of the Eye", p. 121. Livingstone, Edinburgh.
BARTON, A. A., and CAUSEY, G. (1958). *J. Anat. (Lond.)*, **92**, 399.
BEAUFAY, H., and DE DUVE, C. (1959). *Biochem. J.*, **73**, 604.
BECKER, N. H. (1963). In "Selective Vulnerability of the Brain in Hypoxaemia". C.I.O.M.S. Symposium (ed. J. P. SCHADÉ and W. H. MCMENEMEY), p. 317. Blackwell Scientific Publications, Oxford.
BERGSTRAND, A., and BUCHT, H. (1959). *J. Path. Bact.*, **77**, 231.
BLOODWORTH, J. M. B., and MOLITOR, D. L. (1965). *Invest. Ophthal.*, **4**, 1037.
BUBIS, J. J., and LUSE, S. A. (1964). *Amer. J. Path.*, **44**, 299.
BUNGE, R. P., BUNGE, M. B., and RIS, H. (1960). *J. biophys. biochem. Cytol.*, **7**, 685.
CAJAL, S. RAMON Y (1911). "Histologie du système nerveux de l'homme et des vertébrés" (trans. L. AZOULAY), Vol. 2. Maloine, Paris.
——— (1928). "Degeneration and Regeneration of the Nervous System" (trans. R. M. MAY). Oxford University Press, London.
CAULFIELD, J. B., and KLIONSKY, B. (1959). *Amer. J. Path.*, **35**, 489.
CAUSEY, G., and HOFFMAN, H. (1955). *Brit. J. Cancer.*, **9**, 666.
CAVANAGH, J. B. (1954). *J. Neurol. Neurosurg. Psychiat.*, **17**, 163.
CHRISTENSEN, L. (1958). *Trans. Amer. ophthal. Soc.*, **56**, 451.
COGAN, D. G. (1962). *Amer. J. Ophthal.*, **54**, 347.
CONFER, D. B., and STENGER, R. J. (1964). *Amer. J. Path.*, **45**, 533.
CRAMER, F., and ALPERS, B. J. (1932). *Arch. Path.*, **13**, 23.
CUNHA-VAZ, J. G., SHAKIB, M., and ASHTON, N. (1966). *Brit. J. Ophthal.*, **50**. In the press.
D'AGOSTINO, A. N. (1964). *Neurology*, **14**, 114.
DAVISON, P. F., and TAYLOR, E. W. (1960). *J. gen. Physiol.*, **43**, 801.
DAVSON, H., and SPAZIANI, E. (1959). *J. Physiol. (Lond.)*, **149**, 135.
DE DUVE, C. (1963). *Scient. Am.*, **208**, no. 5, p. 64.
——— and BEAUFAY, H. (1959). *Biochem. J.*, **73**, 610.
DE ROBERTIS, E. (1956). *J. biophys. biochem. Cytol.*, **2**, 503.
——— and SOTELO, J. R. (1952). *Exp. Cell Res.*, **3**, 433.
DOLLERY, C. T., HODGE, J. V., and ENGEL, M. (1962). *Brit. med. J.*, **2**, 1210.
———, RAMALHO, P. S., PATERSON, J. W., HILL, D. W., HENKIND, P., SHAKIB, M., and ASHTON, N. (1965). *Lancet*, **1**, 1303.
ESTABLE, C., ACOSTA-FERREIRA, W., and SOTELO, J. R. (1957). *Z. Zellforsch.*, **46**, 387.
EVANS, D. H. L., and GRAY, E. G. (1961). "Cytology of Nervous Tissue", Proc. Anat. Soc. Gt. Britain and Ireland, p. 71. Taylor and Francis, London.
FERRARO, A., and DAVIDOFF, L. M. (1928). *Arch. Path. (Chicago)*, **6**, 1030.
FINE, B. S., and ZIMMERMAN, L. E. (1962). *Invest. Ophthal.*, **1**, 304.
GONATAS, N. K., LEVINE, S., and SHOULSON, R. (1964). *Amer. J. Path.*, **44**, 565.
———, TERRY, R. D., WINKLER, R., KOREY, S. R., GOMEZ, C. J., and STEIN, A. (1963). *J. Neuropath. exp. Neurol.*, **22**, 557.

GRAY, E. G. (1964). "Electron Microscopic Anatomy" (ed. S. M. KURTZ), p. 377. Academic Press, New York.
——— and HAMLYN, L. H. (1962). *J. Anat.* (*Lond.*), **96**, 309.
GUILLERY, R. W. (1965). In "Degeneration Patterns in the Nervous System", *Progress in Brain Research* (ed. M. SINGER and J. P. SCHADÉ) **14**, 57. Elsevier, Amsterdam.
HAGER, H. (1963). In "Selective Vulnerability of the Brain in Hypoxaemia", p. 125. C.I.O.M.S. Symposium (ed. J. P. SCHADÉ and W. H. MCMENEMEY). Blackwell Scientific Publications, Oxford.
HAIN, R. F. (1963). *J. Neuropath. exp. Neurol.*, **22**, 327.
HARTMANN, J. F. (1954). *Anat. Rec.*, **118**, 19.
——— (1958). In "Biology of Neuroglia" (ed. W. F. WINDLE), p. 50. Thomas, Springfield, Ill.
HELANDER, H. F. (1961). *J. Ultrastruct. Res.*, **5**, 257.
HILLS, C. P. (1964). *Amer. J. Path.*, **44**, 531.
HOGAN, M. J., and FEENEY, L. (1963). *J. Ultrastruct. Res.*, **9**, 47.
——— and ZIMMERMAN, L. E. (1962). "Ophthalmic Pathology", 2nd ed., p. 90. Saunders, Philadelphia.
HUDSON, G., and HARTMANN, J. F. (1961). *Z. Zellforsch. mikrokop. Anat.*, **54**, 147.
HYDÉN, H. (1960). In "The Cell" (ed. J. BRACHET and A. E. MIRSKY), **4**, 297. Academic Press, New York.
ITO, S. (1962). In "Fifth International Congress for Electron Microscopy" (ed. S. S. BREESE Jr), **2**, L–5. Academic Press, New York.
JABLONSKI, W., and MEYER, H. (1938). *J. Anat.*, **73**, 130.
JIMÉNEZ DE ASÚA, F. (1927). *Z. ges. Neurol. Psychiat.*, **109**, 354.
JOHNSON, A. C., MCNABB, A. R., and ROSSITER, R. J. (1950). *Arch. Neurol. Psychiat.* (*Chicago*), **64**, 105.
KARLSSON, U., and SCHULTZ, R. L. (1966). *J. Ultrastruct. Res.*, **14**, 47.
KARRER, H. E. (1960). *J. biophys. biochem. Cytol.*, **7**, 357.
KONIGSMARK, B. W., and SIDMAN, R. L. (1963). *J. Neuropath. exp. Neurol.*, **22**, 643.
LAMPERT, P., BLUMBERG, J. M., and PENTSCHEW, A. (1964). *J. Neuropath. exp. Neurol.*, **23**, 60.
——— and CARPENTER, S. (1965). *Ibid.*, **24**, 11.
——— and PENTSCHEW, A. (1964). *Acta neuropath.* (*Berl.*), **4**, 158.
LEE, J. C. (1963). *J. comp. Neurol.*, **120**, 65.
LUSE, S. A. (1958). *Lab. Invest.*, **7**, 401.
——— and SMITH, K. R., Jr. (1964). *Amer. J. Path.*, **44** 553.
LUBIŃSKA, L. (1964). In "Mechanisms of Neural Regeneration", *Progress in Brain Research* (ed. M. SINGER and J. P. SCHADÉ), **13**, 1. Elsevier, Amsterdam.
MACDONALD, M. K., and IRELAND, J. T. (1964). In CIBA Foundation "Colloquia on Endocrinology", (ed. M. P. CAMERON and M. O'CONNOR), **15**, 301. Churchill, London.
MCGEE, W. G., and ASHWORTH, C. T. (1963). *Amer. J. Path.*, **43**, 273.
MAJNO, G., and KARNOVSKY, M. L. (1961). *J. Neurochem.*, **8**, 1.
MISSOTTEN, L. (1965). "The Ultrastructure of the Human Retina", p. 131. Arscia, Brussels.
MÖLBERT, E., and GUERRITORE, D. (1957). *Beitr. path. Anat.*, **117**, 32.
MÜLLER, H. (1858). *Albrecht v. Graefes Arch. Ophthal.*, **4**, 363.
OHMI, S. (1961). *Z. Zellforsch.*, **54**, 39.
PALAY, S. L. (1958). In "Biology of Neuroglia" (ed. W. F. WINDLE), p. 24. Thomas, Springfield, Ill.
——— and PALADE, G. E. (1955). *J. biophys. biochem. Cytol.*, **1**, 69.
PARKER, G. H. (1929). *Quart. Rev. Biol.*, **4**, 155.
POLYAK, S. L. (1941). "The Retina". University Press, Chicago.
RALL, D. P., OPPELT, W. W., and PATLAK, C. S. (1962). *Life Sci.*, no. 2, p. 43.
REES, S. B., CAMERINI-DAVALOS, R. A., CAULFIELD, J. B., LOZANO-CASTANEDA, O., CERVANTES-AMEZCUS, A., TATON, J., POMETTA, D., KRAUTHAMMER, J. P., and MARBLE, A. (1964). In CIBA Foundation "Colloquia on Endocrinology" (ed. M. P. CAMERON and M. O'CONNOR), **15**, 315. Churchill, London.
REINECKE, R. D., KUWABARA, T., COGAN, D. G., and WEIS, D. R. (1962). *Arch. Ophthal.* (*Chicago*), **67**, 470.
RIO-HORTEGA, P. DEL (1921). *Arch. Neurobiol.* (*Madr.*), **2**, 212.
ROBERTSON, D. M., and VOGEL, F. S. (1962). *J. Cell Biol.*, **15**, 313.
ROBERTSON, J. D., BODENHEIMER, T. S., and STAGE, D. E. (1963). *Ibid.*, **19**, 159.
SAMUELS, S., GONATAS, N. K., and WEISS, M. (1965). *J. Neuropath. exp. Neurol.*, **24**, 256.
SCHLAEPFER, W. W., and HAGER, H. (1964). *Amer. J. Path.*, **45**, 423.
SCHLOTE, W. (1964). *Acta neuropath.* (*Berl.*), **4**, 138.
SCHMITT, F. O. (1961). "Actualités neurophysiologiques", 3rd ser. (ed. A. M. MONNIER), p. 355. Paris, Masson.
SCHULTZ, R. L. (1964). *J. comp. Neurol.*, **122**, 281.
——— and PEASE, D. C. (1959). *Amer. J. Path.*, **35**, 1017.
SHAKIB, M. (1966a). Unpublished data.
——— (1966b). Personal observation.

SJÖSTRAND, F. S. (1960). In "Modern Scientific Aspects of Neurology" (ed. J. N. CUMINGS), p. 188. Arnold, London.
——— (1962). In "The Interpretation of Ultrastructure" (ed. R. J. C. HARRIS), p. 47. Academic Press, New York.
SMITH, K. R. (1961). *J. comp. Neurol.*, **116**, 103.
STEINER, J. W., MIYAI, K., and PHILLIPS, M. J. (1964). *Amer. J. Path.*, **44**, 169.
STOECKENIUS, W. (1962). In "The Interpretation of Ultrastructure" (ed. R. J. C. HARRIS), p. 349. Academic Press, New York.
TAXI, J. (1959). *C.R. Acad. Sci. (Paris)*, **248**, 2796.
TERRY, R. D., and PEÑA, C. (1965). *J. Neuropath. exp. Neurol.*, **24**, 200.
——— and WEISS, M. (1963). *Ibid.*, **22**, 18.
TRIER, J. S. (1958). *J. biophys. biochem. Cytol.*, **4**, 13.
VAN HARREVELD, A., CROWELL, J., and MALHOTRA, S. K. (1965). *J. Cell. Biol.*, **25**, 117.
VIAL, J. D. (1958). *J. biophys. biochem. Cytol.*, **4**, 551.
WALBERG, F. (1963). *Exp. Neurol.*, **8**, 112.
WALKER, B. E. (1960). *J. Ultrastruct. Res.*, **3**, 345.
WEBSTER, H. (1962). *J. Cell Biol.*, **12**, 361.
WELLS, A. Q., and CARMICHAEL, E. A. (1930). *Brain*, **53**, 1.
WIENER, J., SPIRO, D., and LATTES, R. G. (1965). *Amer. J. Path.*, **47**, 457.
WOLTER, J. R. (1955). *Amer. J. Ophthal.*, **40**, no. 5, pt 2, p. 88.
——— (1957). *Ibid.*, **43**, 885.
——— (1959a). *Ibid.*, **48**, no. 5, pt 2, p. 370.
——— (1959b). *Ibid.*, **48**, 473.
YAMASHITA, T., and ROSEN, D. A. (1962). *Arch. Ophthal. (Chicago)*, **67**, 785.
ZEMAN, W. (1963). In "Selective Vulnerability of the Brain in Hypoxaemia" C.I.O.M.S. Symposium (ed. J. P. SCHADÉ and W. H. MCMENEMEY), p. 327. Blackwell Scientific Publications, Oxford.

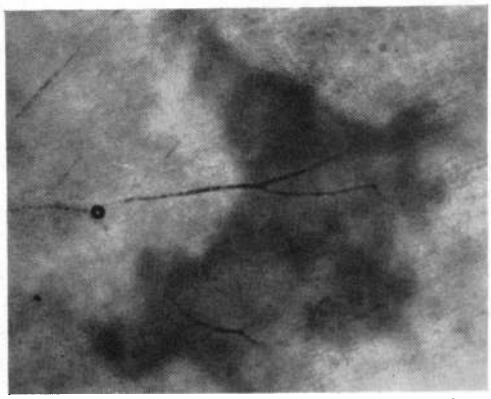

FIG. 1.—Flat preparation of fixed retina 3 days after embolization shows glass microsphere in arteriole and ischaemic lesion distal to it. ×45.

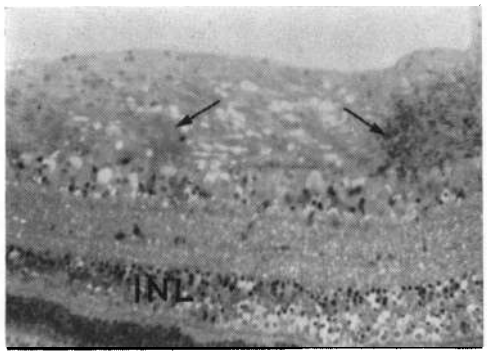

FIG. 3.—Retina 5 hours after embolization showing densely staining granules on both sides of the vacuolated area in the NFL (arrows). Note clear cells in the inner nuclear layer (INL). × 113.

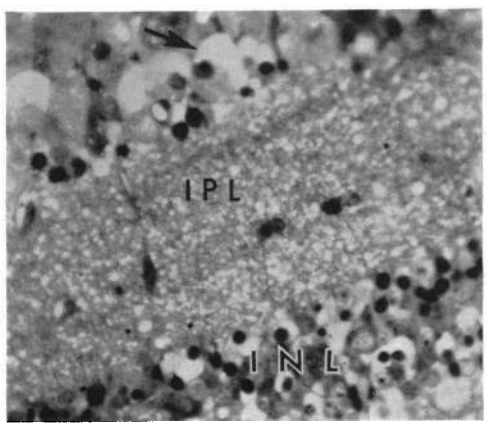

FIG. 5.—High-power view of Fig. 3 showing condensed chromatin and clear areas (arrow) in the ganglion cells. Inner plexiform layer (IPL) is vesicular. Inner nuclear layer (INL) contains clear cells with dark or pale nuclei. × 300.

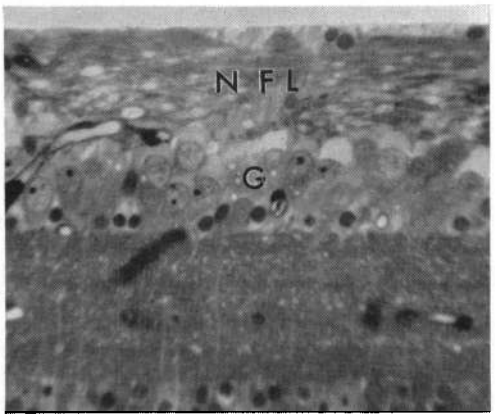

FIG. 2.—Nerve fibre layer (NFL) one hour after embolization showing oval vacuoles and some ganglion cells (G) with clear areas in their cytoplasm. × 300.

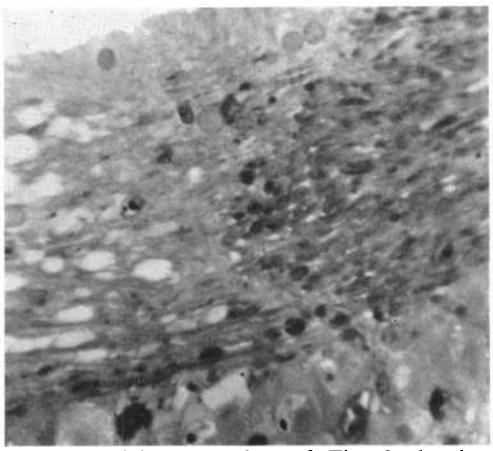

FIG. 4.—High-power view of Fig. 3 showing vacuoles to be swollen axons. Densely staining granules fill the less expanded axons. × 300.

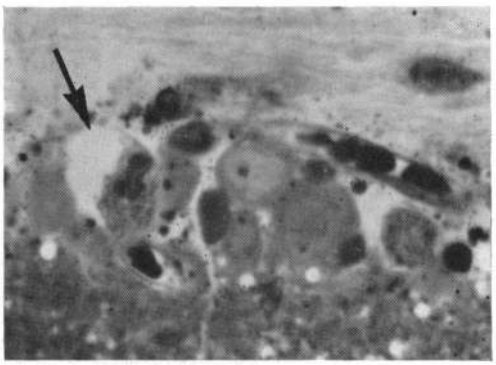

FIG. 6.—Retina 24 hours after embolization showing degenerated ganglion cells with cystic cavities in their cytoplasm (arrow) and dark, irregular nuclei on one side. × 520.

Figs 2–6: Epon sections. Toluidine blue stain.

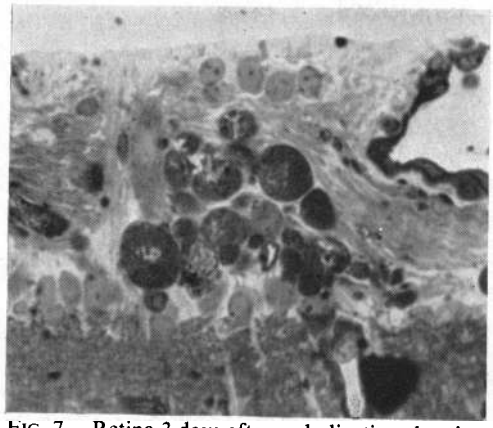

FIG. 7.—Retina 3 days after embolization showing basophilic round or oval bodies in the nerve fibre layer. × 460.

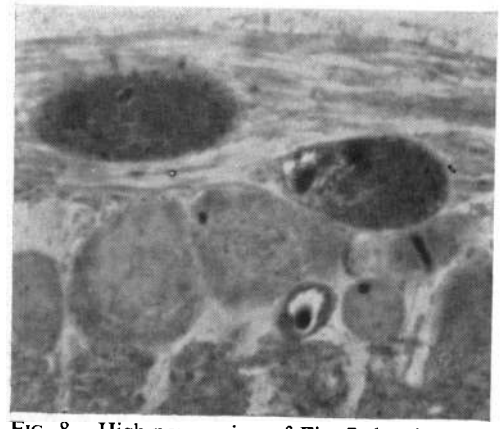

FIG. 8.—High-power view of Fig. 7 showing that the bodies are swollen hypertrophic axons. × 1,160.

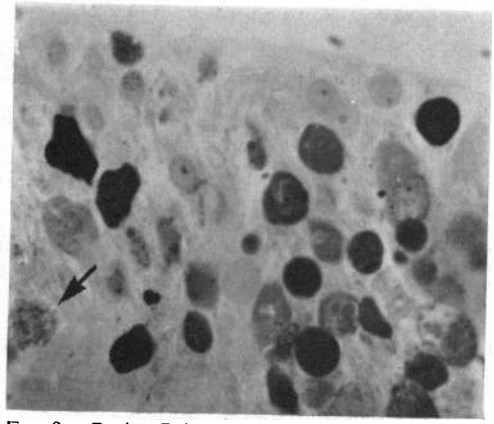

FIG. 9.—Retina 7 days after embolization showing round or oval bodies becoming either dense or vesicular (arrow). × 350.

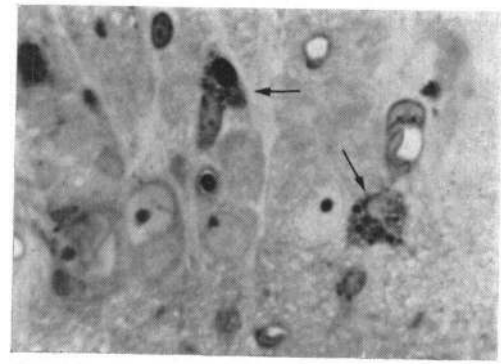

FIG. 10.—Retina 14 days after embolization. Granular macrophages (arrows) are seen around blood vessels. × 520.

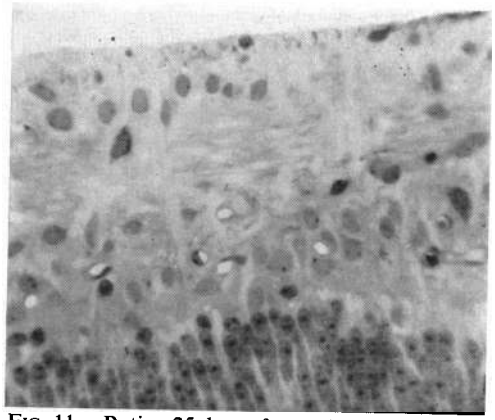

FIG. 11.—Retina 25 days after embolization showing atrophy and disorganization in the inner layers of the retina with glial replacement. × 300.

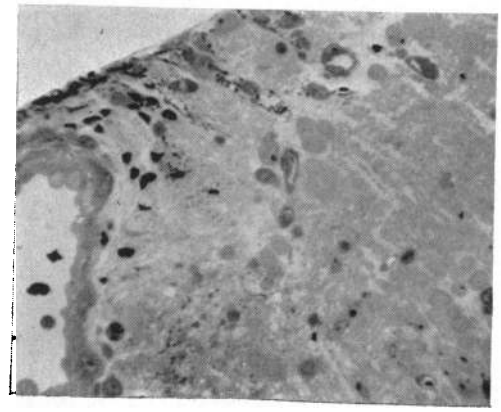

FIG. 12.—Haemorrhagic area in retina. Red cells, fibrogranular deposits, and leucocytes are scattered throughout the inner layers. × 300.

Figs 7–12: Epon sections. Toluidine blue stain.

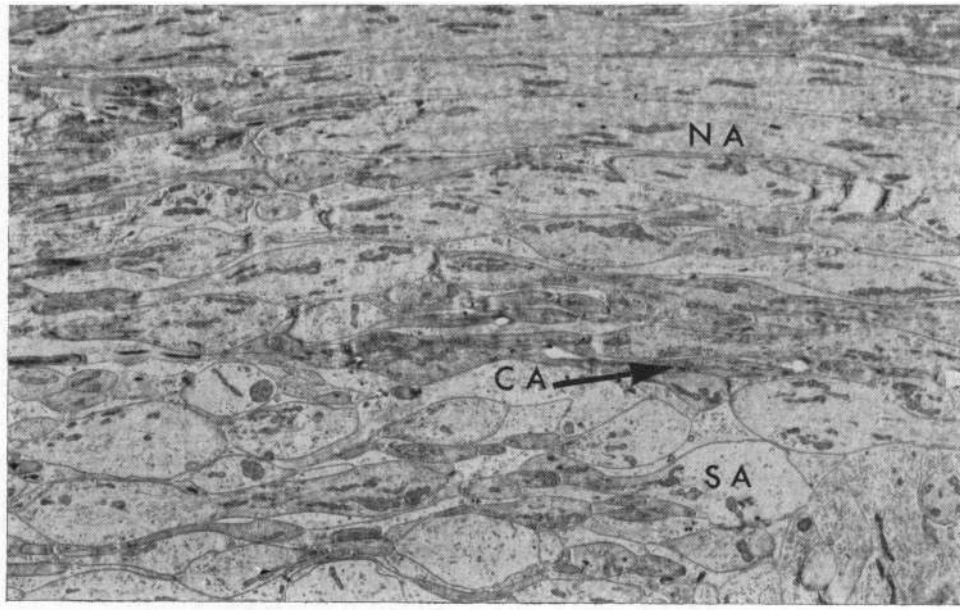

FIG. 13.—One-hour specimen. Swollen axons (SA) are scattered amongst many normal axons (NA) or axons compressed by swelling (CA). EM × 2,700.

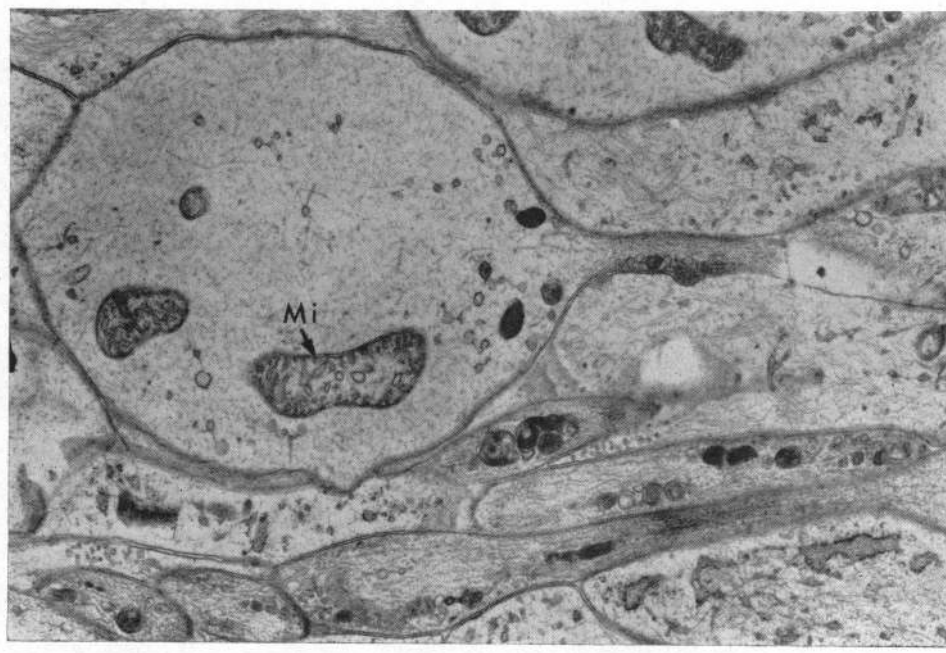

FIG. 14.—Higher magnification of swollen segment of axon showing fine granular material, sparse neurofilaments, and a few mitochondria (Mi). EM × 15,300.

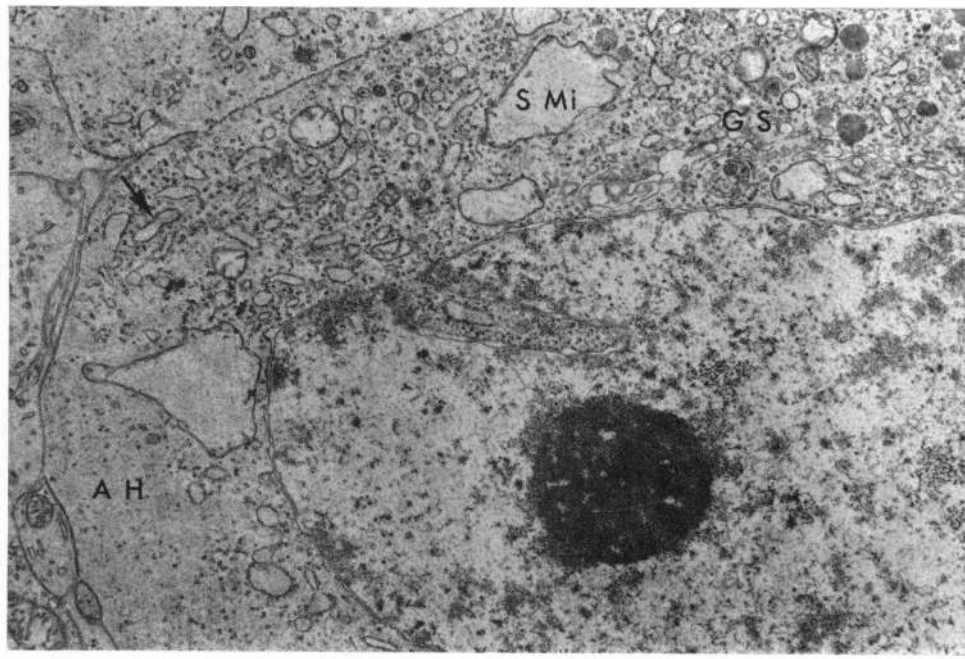

FIG. 15.—One-hour specimen. Ganglion cell showing swollen mitochondria (S Mi), occasional short broken cristae, dilated endoplasmic reticulum (arrow), a pronounced Golgi system (GS), and slight clumping of the nuclear chromatin. Note the loss of organelles at the side of axonal hillock (AH). EM × 11,200.

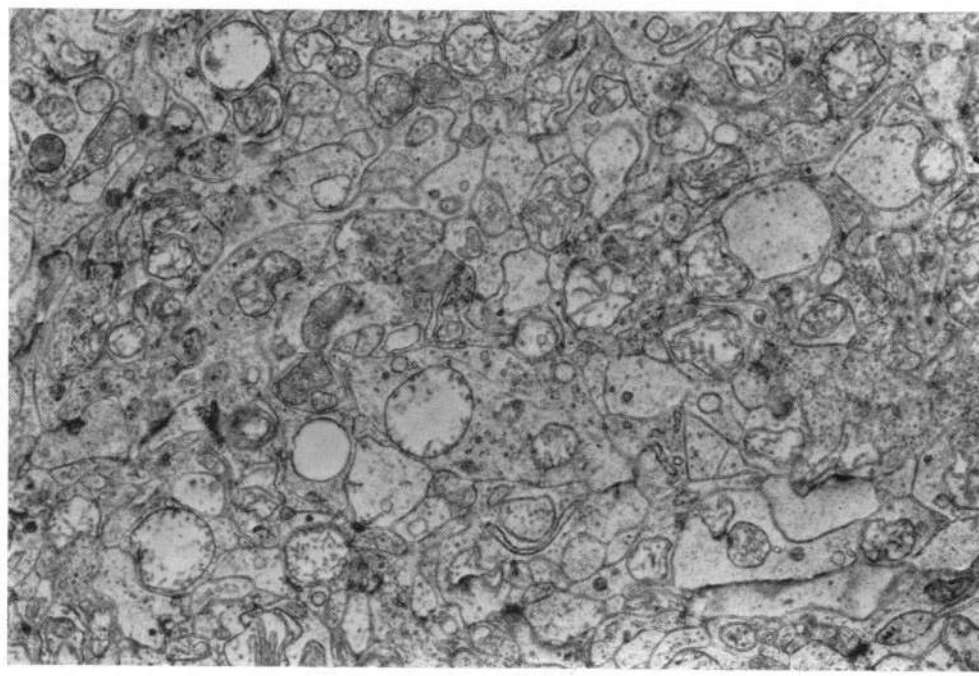

FIG. 16.—One-hour specimen. Swollen mitochondria are present in axons and dendrites of the inner plexiform layer. EM × 11,000.

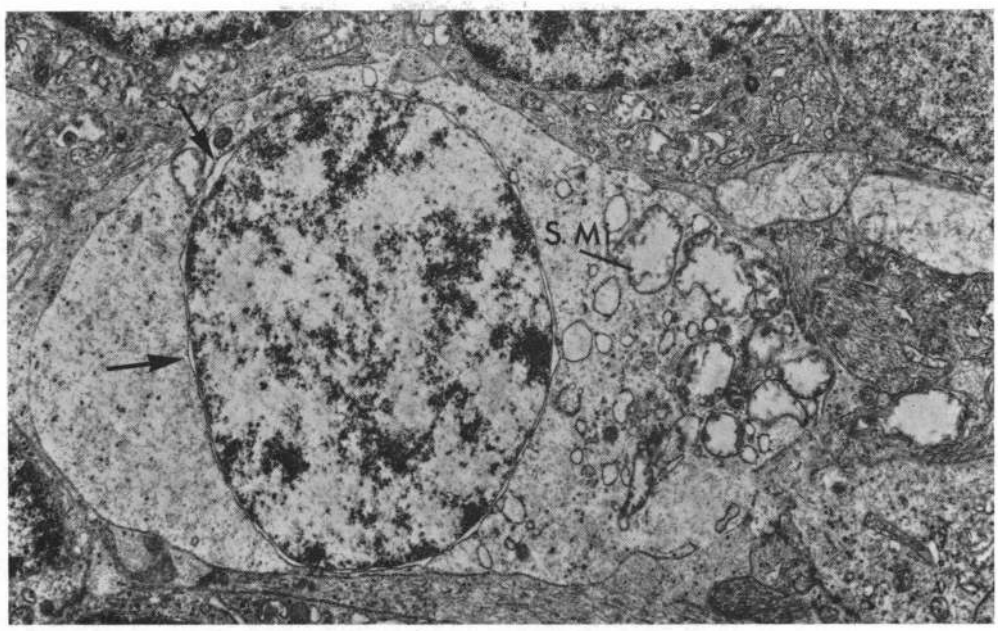

FIG. 17.—One-hour specimen. Cell in the inner nuclear layer showing clear cytoplasm, swollen mitochondria (S Mi) and vacuolated endoplasmic reticulum. Nuclear chromatin is clumped and wide gaps (arrows) are present between the nuclear membranes. EM × 8,400.

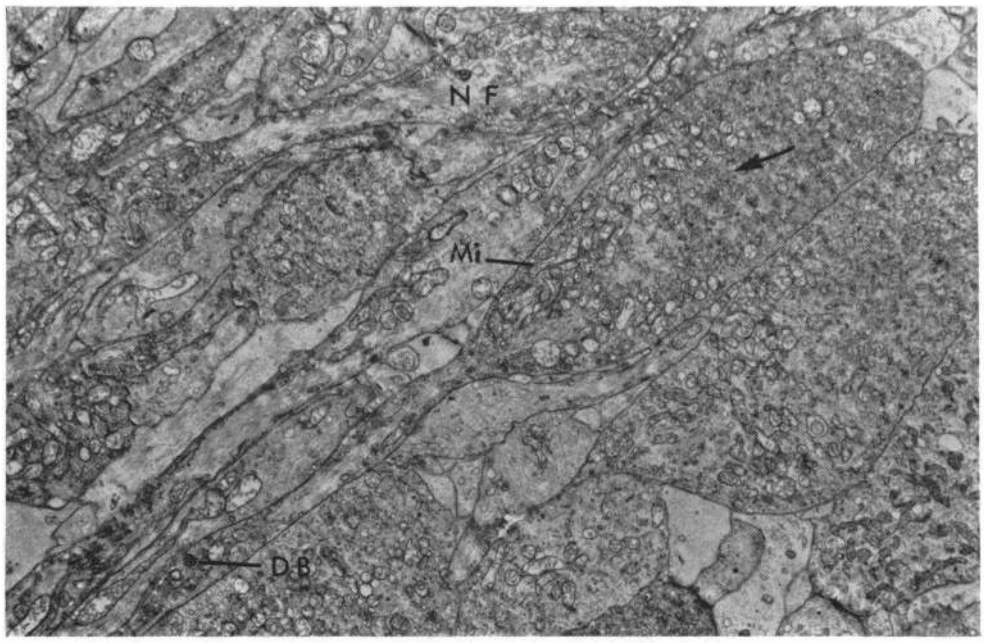

FIG. 18.—Five-hour specimen. Longitudinal section of affected axons showing swollen segments filled with membrane-bound microvesicles (arrow), tubules, mitochondria (Mi), a few dense bodies (DB), and indistinct neurofilaments (NF). Areas between swollen segments have a relatively normal structure. EM × 4,300.

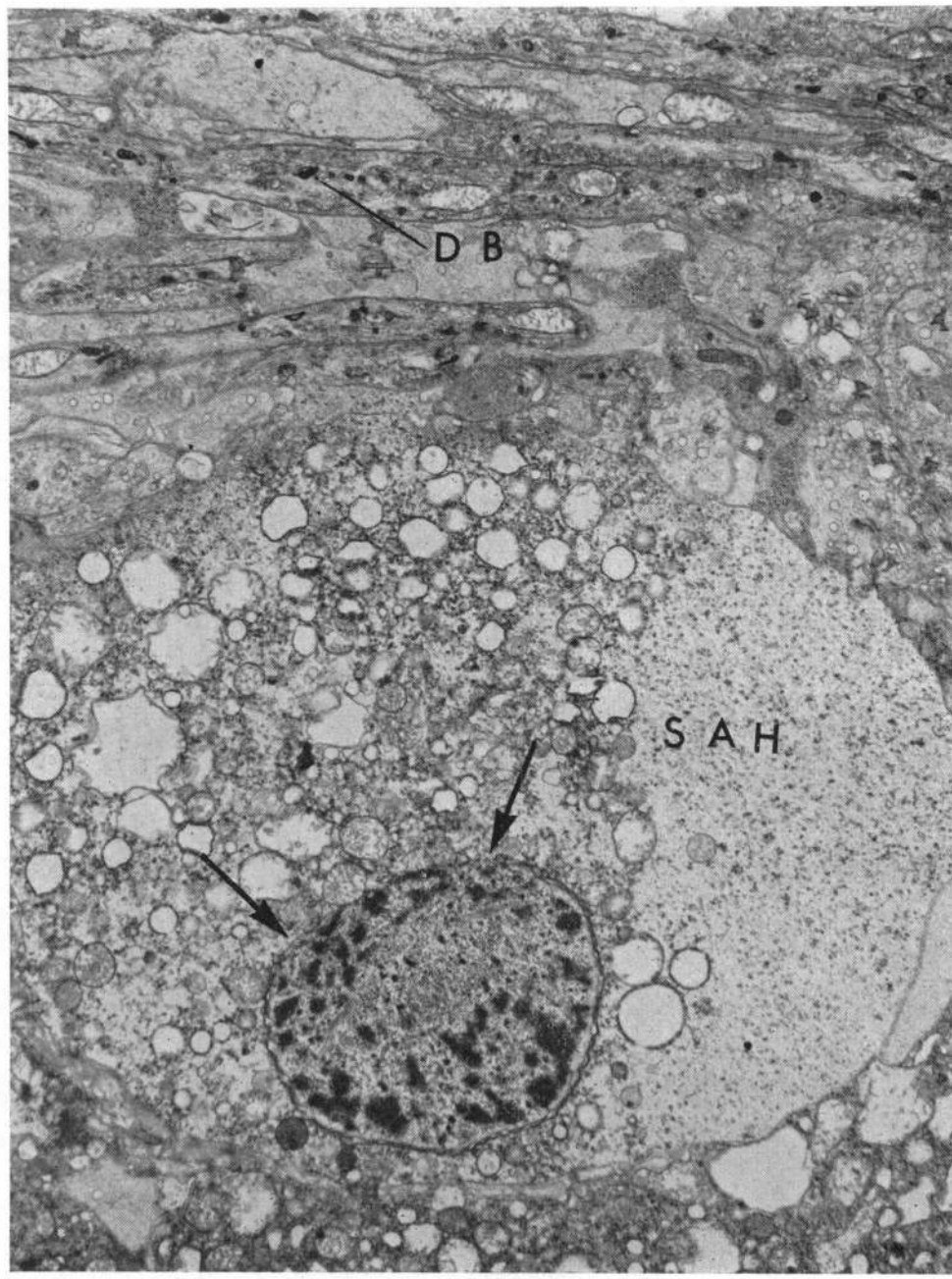

FIG. 19.—Five-hour specimen. Degenerated ganglion cell with grossly abnormal organelles, absent at the side of axonal hillock (SAH). Nuclear chromatin is clumped and the nuclear membrane is ruptured (arrows). In the nerve fibres above a number of dense bodies are seen (DB). EM × 7,500.

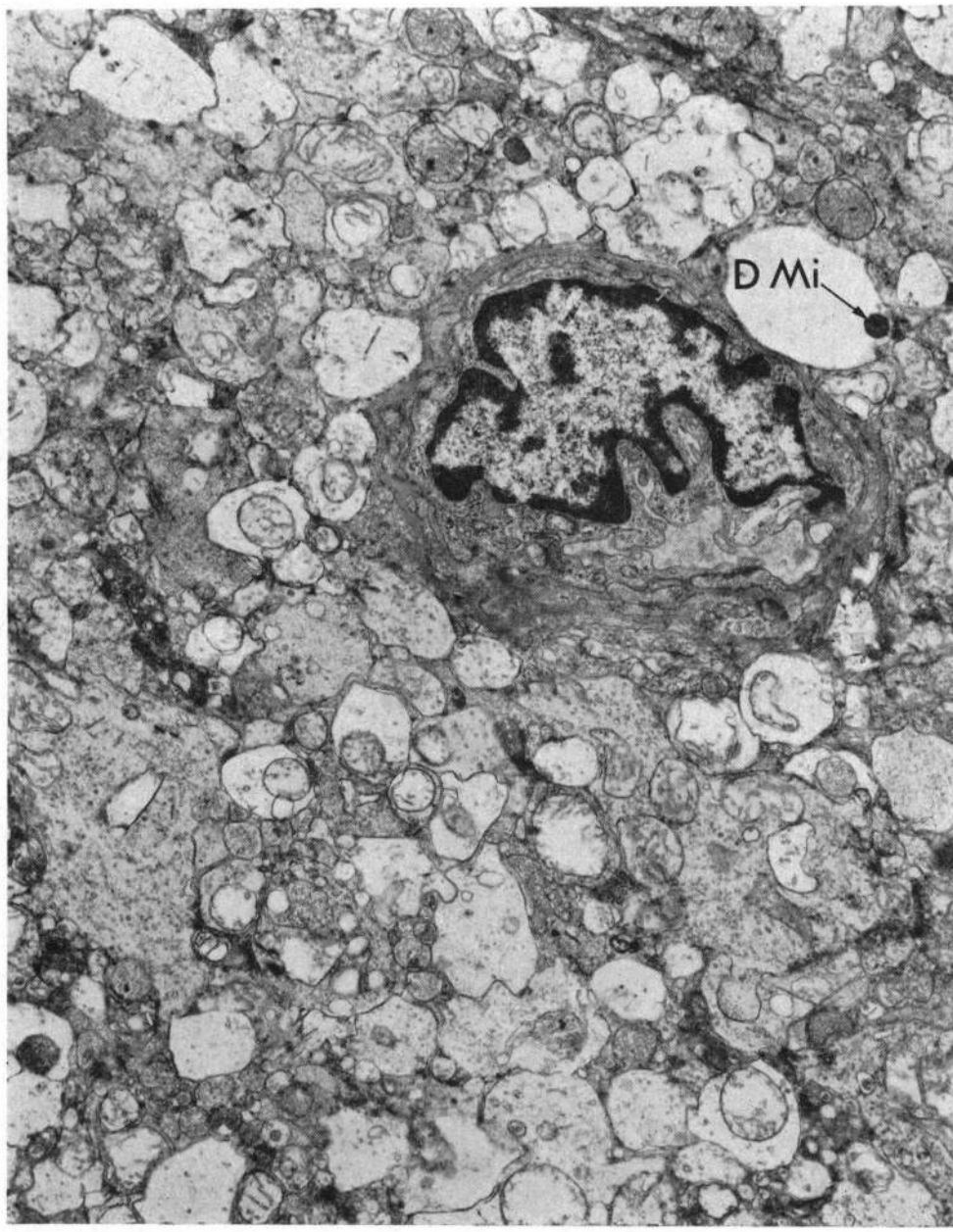

FIG. 20.—Five-hour specimen. Inner plexiform layer. Most mitochondria are swollen in the expanded axons and dendrites, a few appear dense (D Mi). The capillary is almost entirely closed and the endothelial cell is irregular. EM × 12,000.

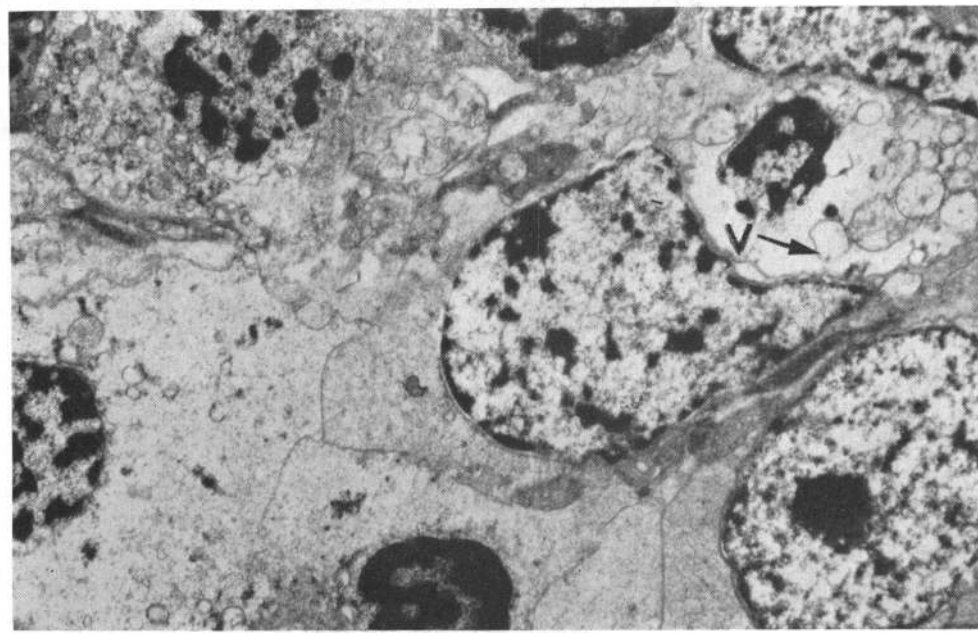

FIG. 21.—Five-hour specimen. Degenerating cells in the inner nuclear layer showing a fragmented cytoplasmic matrix containing disintegrated mitochondria and vacuoles (V). The nuclear membrane is partially disrupted and the chromatin clumped. EM × 7,500.

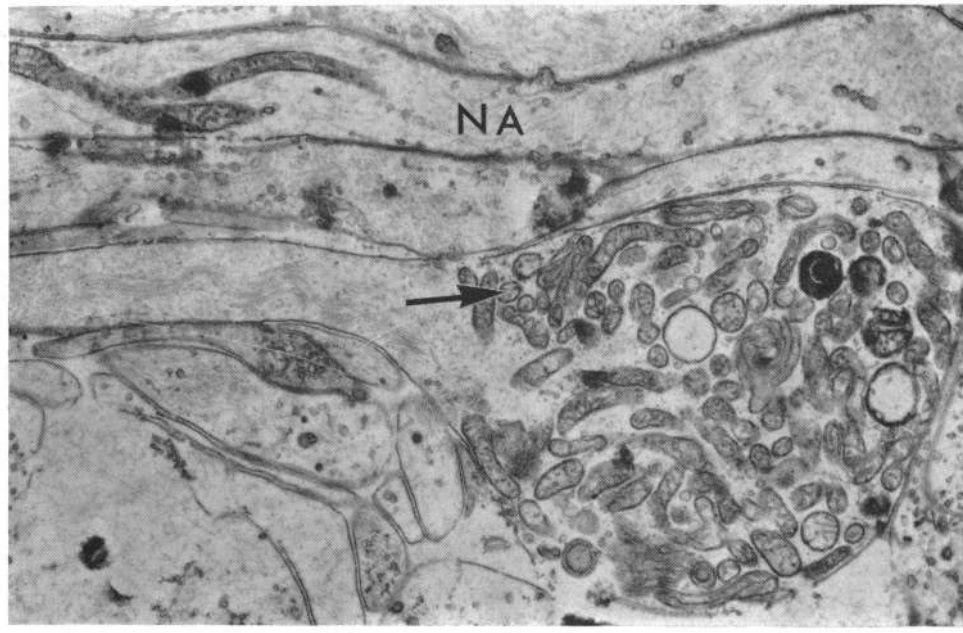

FIG. 22.—Twenty-four-hour specimen. Enlarged axon showing numerous mitochondria, few microvesicular bodies (arrow), dense bodies, and membranous whorls. NA, normal axon. EM × 15,400.

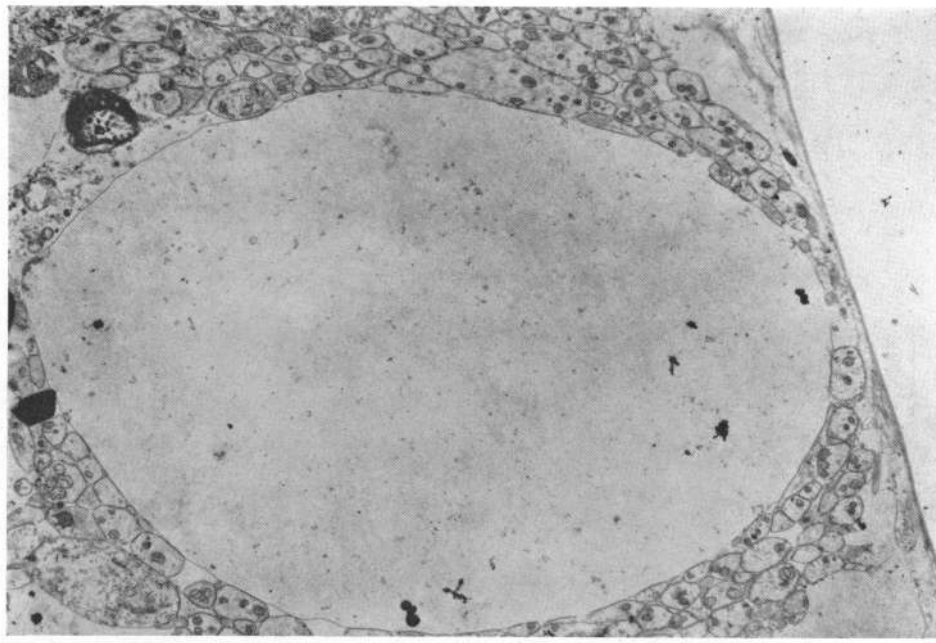

FIG. 23.—Twenty-four-hour specimen. A large membrane-bound vacuole, probably a swollen axon, in the nerve fibre layer. EM ×2,800.

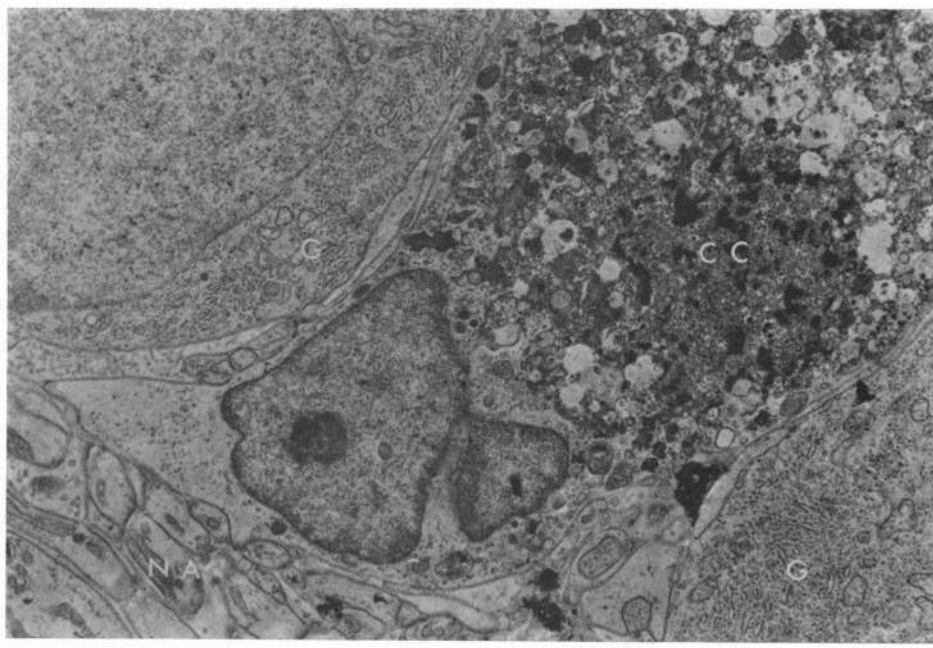

FIG. 24.—Twenty-four-hour specimen. Between two relatively normal appearing ganglion cells (G) a macrophage containing a degenerated cell (probably a ganglion cell) can be seen. Note the coagulated chromatin (CC) on one side, NA, normal axon. EM × 7,460.

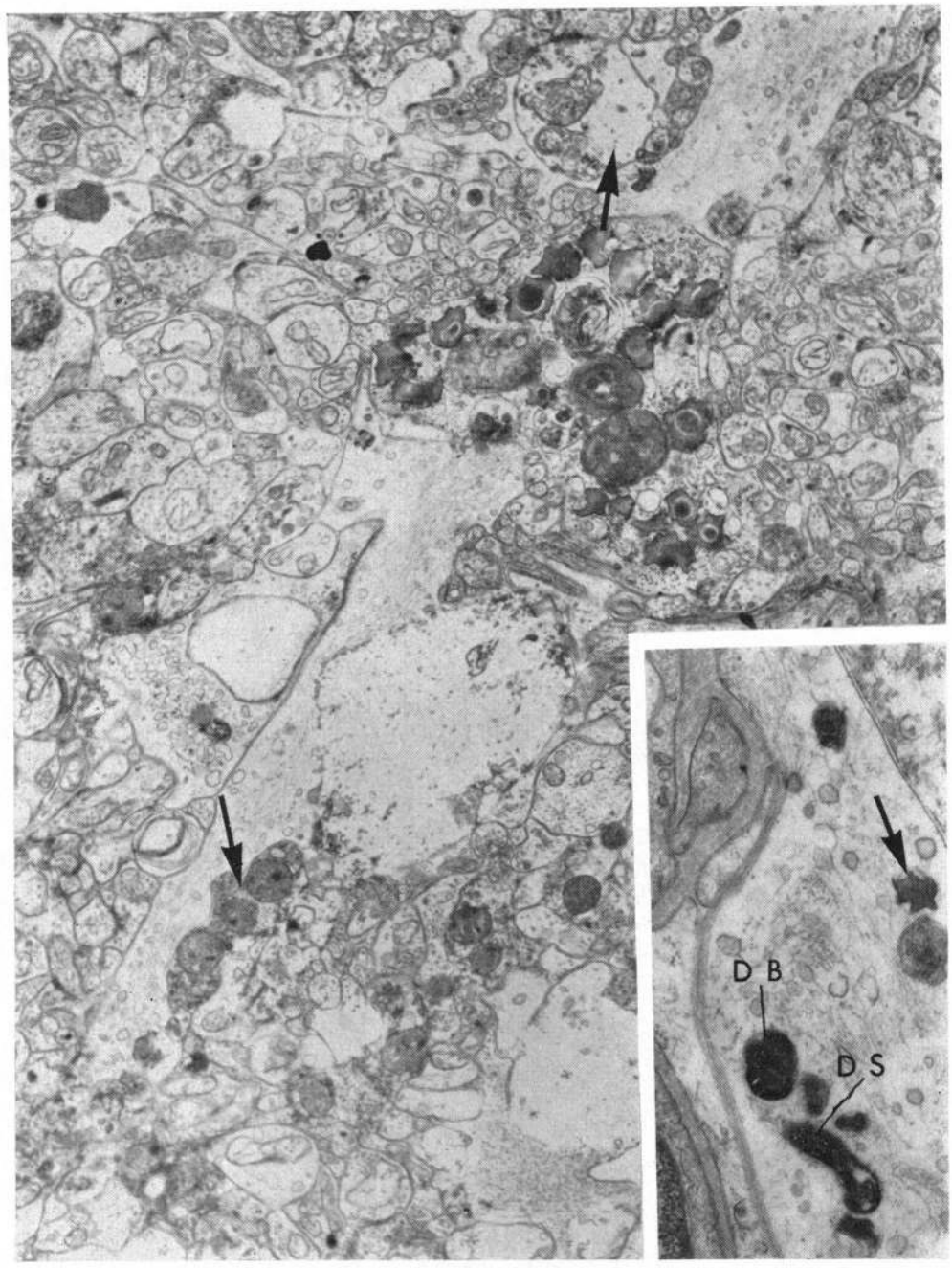

FIG. 25.—Twenty-four-hour specimen. Inner plexiform layer showing swollen or shrunken axons (arrows) and dendrites with disrupted membranes. EM × 13,000.
Inset. High power showing lipid droplets (arrow), dense bodies (DB), and dense structures (DS) resembling mitochondria in glial fibre. EM × 26,000.

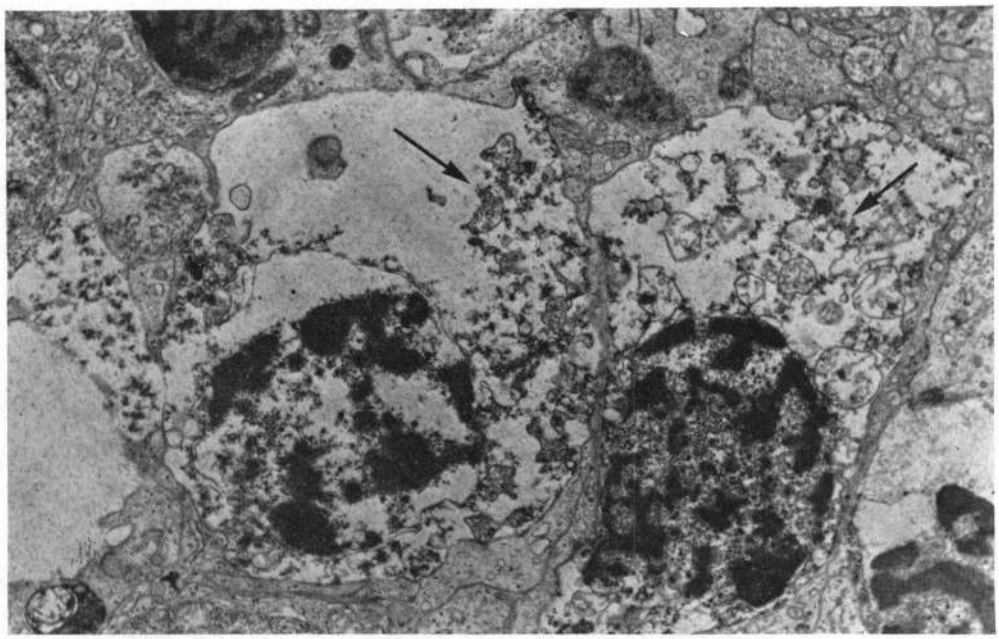

FIG. 26.—Twenty-four-hour specimen. Degenerating cells in the inner nuclear layer containing fragmented cytoplasmic organelles (arrows) and clumps of nuclear chromatin. EM × 7,460.

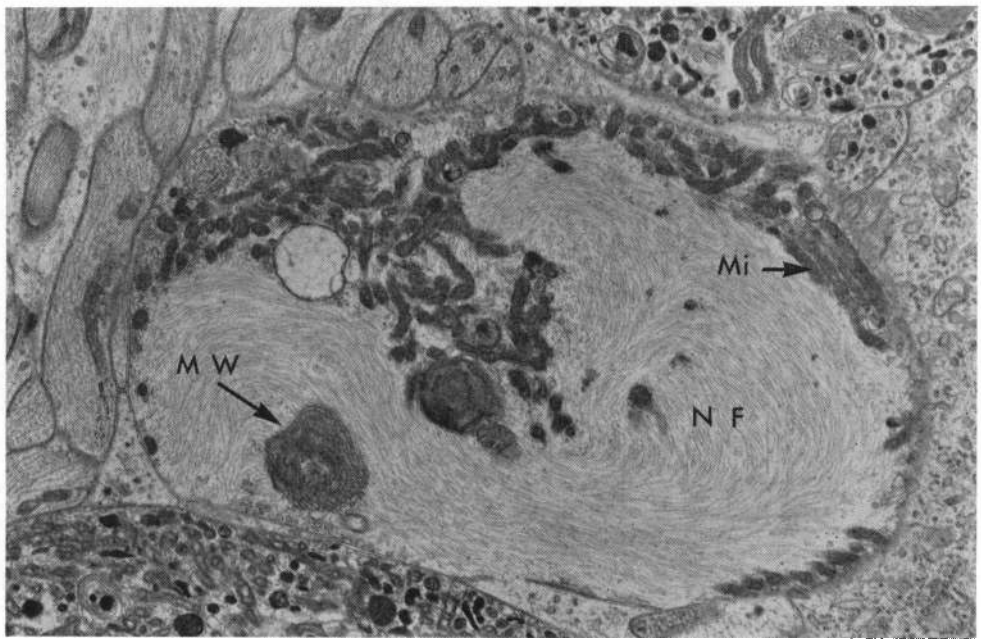

FIG. 27.—Three-day specimen. Enlarged hypertrophic axon packed with bundles of neurofilaments (NF) which have displaced other axonal contents towards the axolemma. Mi, mitochondria; MW, membranous whorl. EM × 12,600.

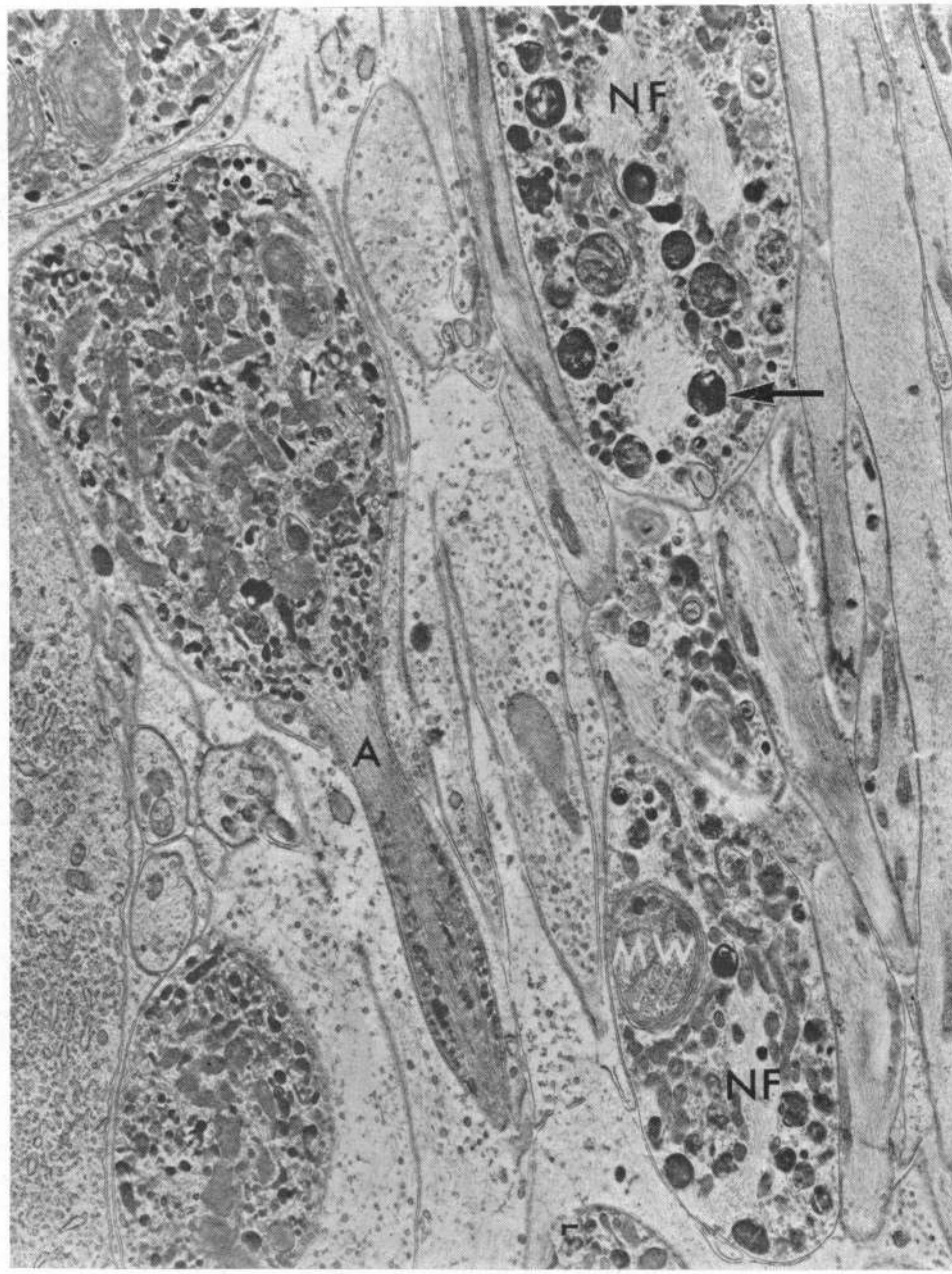

FIG. 28.—Three-day specimen. In one hypertrophic axon (A) dense elongated mitochondria are prominent, while in other hypertrophic axons bundles of neurofilaments (NF) and dense bodies (arrow) predominate. MW, membranous whorl. EM × 13,500.

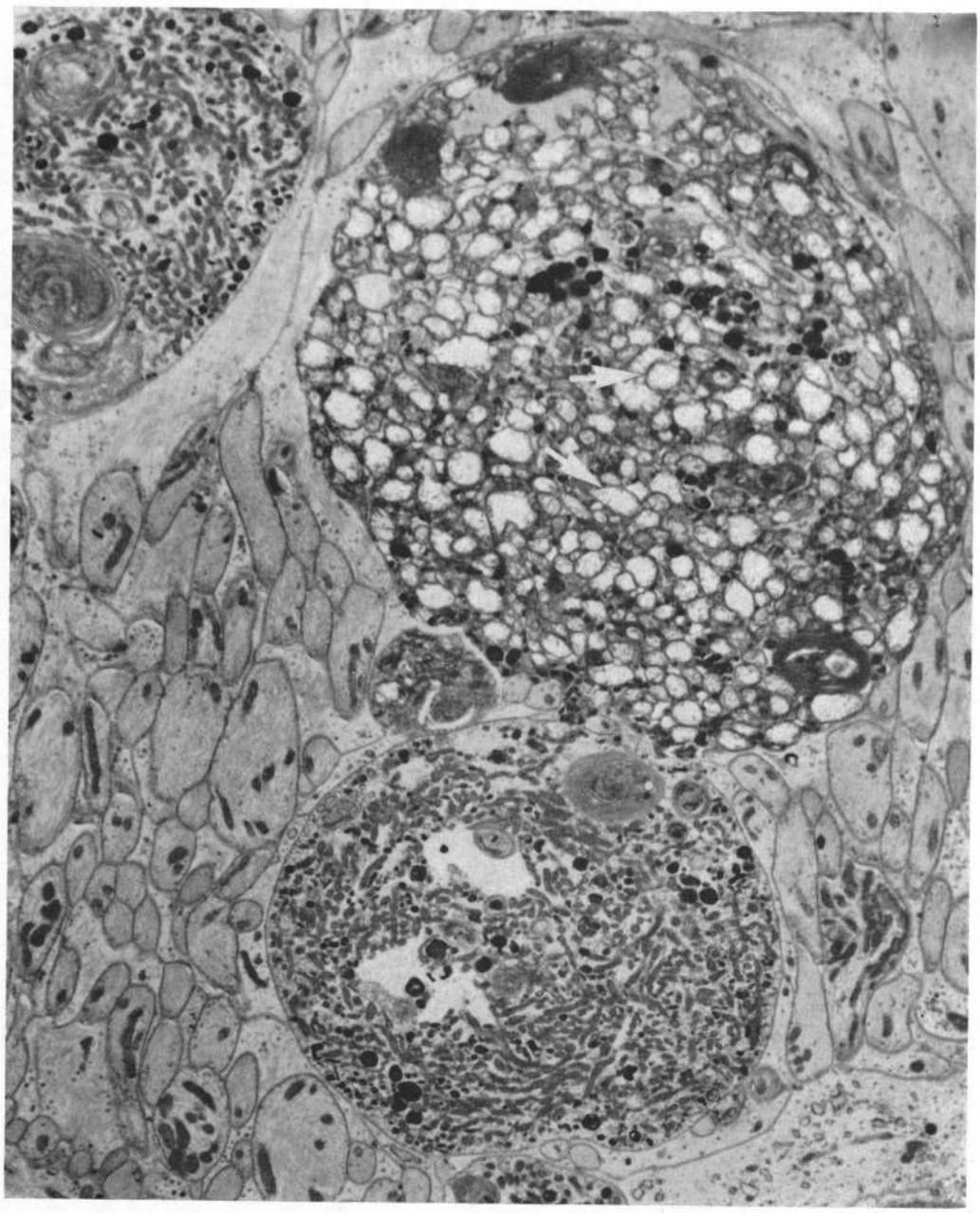

FIG. 29.—Three-day specimen. An axon with numerous swollen mitochondria (arrows) is seen above an axon with a large number of contracted mitochondria. EM × 6,700.

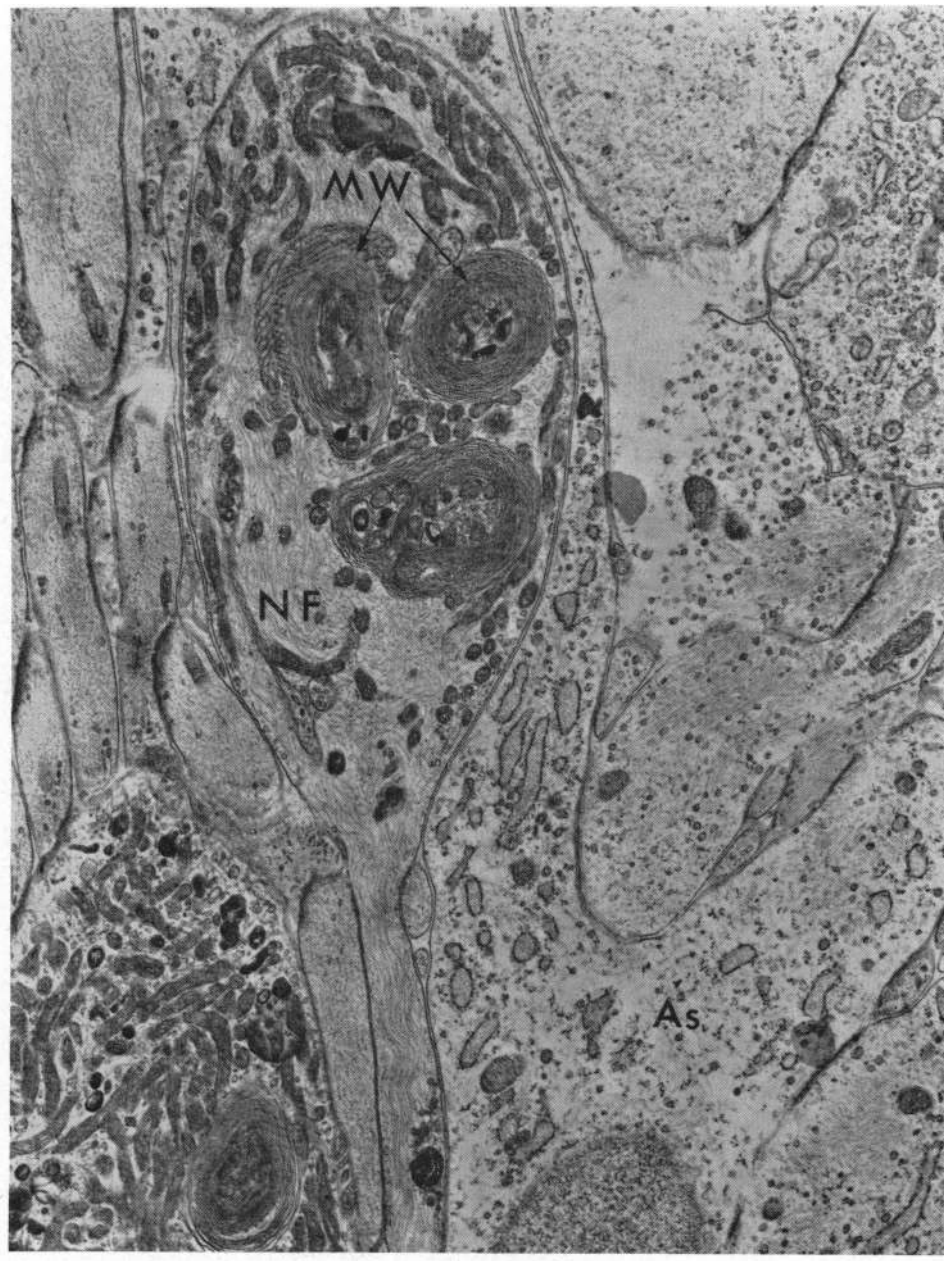

FIG. 30.—Three-day specimen. Swollen hypertrophic axon with bundles of neurofilaments (NF), dense elongated mitochondria, and elaborate membranous whorls (MW). The surrounding astrocyte (As) shows abundant ribosomes and enlarged rough-surfaced endoplasmic reticulum. EM × 15,000.

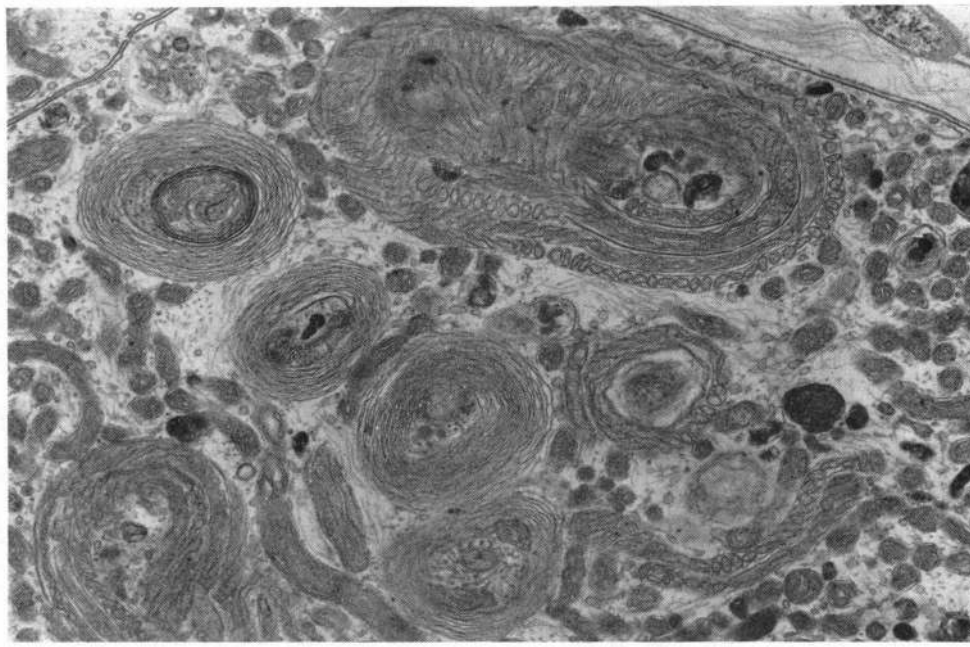

FIG. 31.—Three-day specimen. Several types of membranous whorls seen amongst mitochondria and dense bodies in an axon. EM × 24,200.

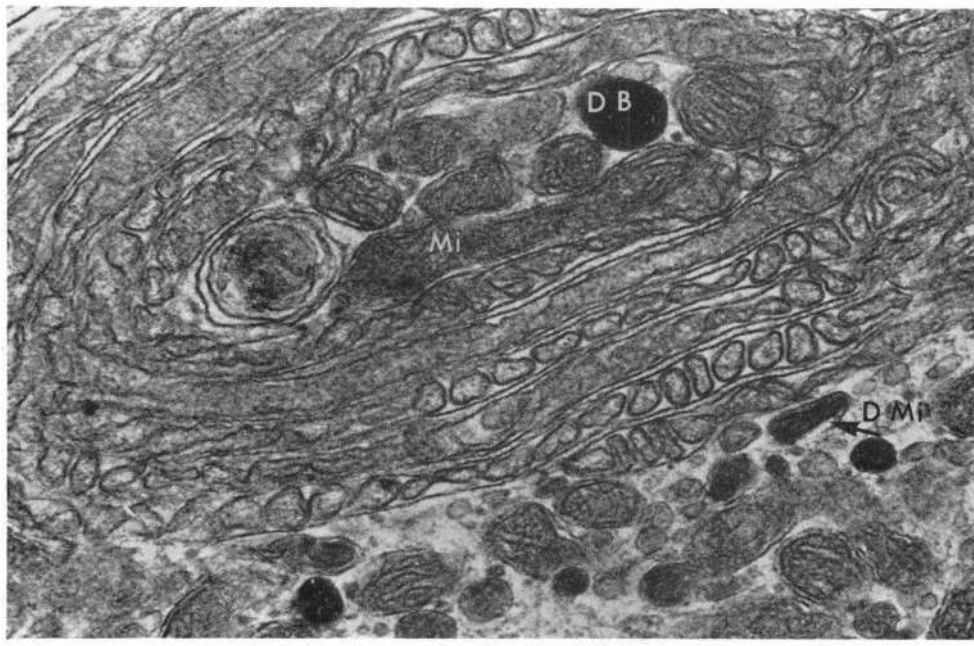

FIG. 32.—Three-day specimen. High magnification of membranous whorl showing elongated, irregular membrane-bound structures between the concentric layers. Centrally a group of cytoplasmic organelles are seen. Mi, mitochondria; DB, dense body; D Mi, a dense body possibly arising from a mitochondria. EM × 66,600.

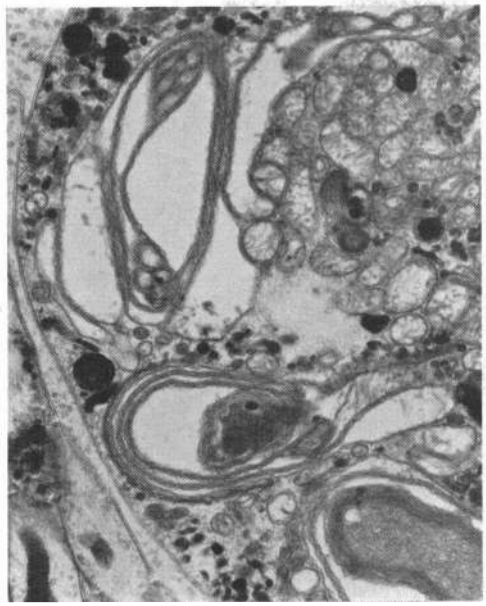

FIG. 33.—Three-day specimen. Compressed concentric membranous whorls in a degenerating axon. EM × 12,600.

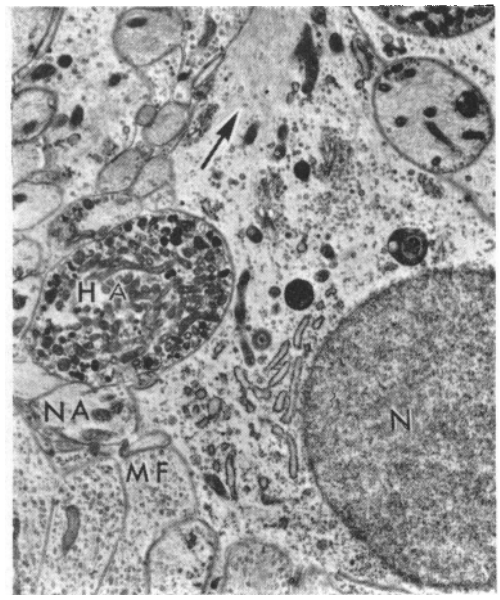

FIG. 34.—Three-day specimen. A hypertrophic axon (HA) surrounded by a reactive astrocyte containing many ribosomes, endoplasmic reticulum and tonofibrils (arrow). NA, normal axon; N, nucleus of the astrocyte; MF, Müller fibre. EM × 8,000.

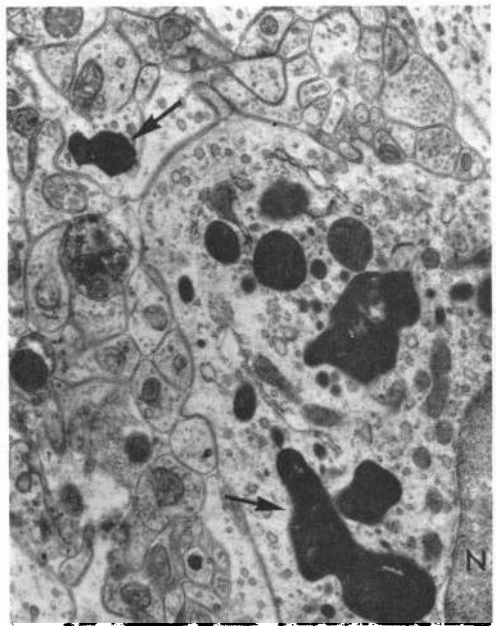

FIG. 35.—Three-day specimen. Inner plexiform layer showing macrophage containing dense material (arrow) similar to that seen outside (arrow). N, nucleus of the macrophage. EM × 14,000.

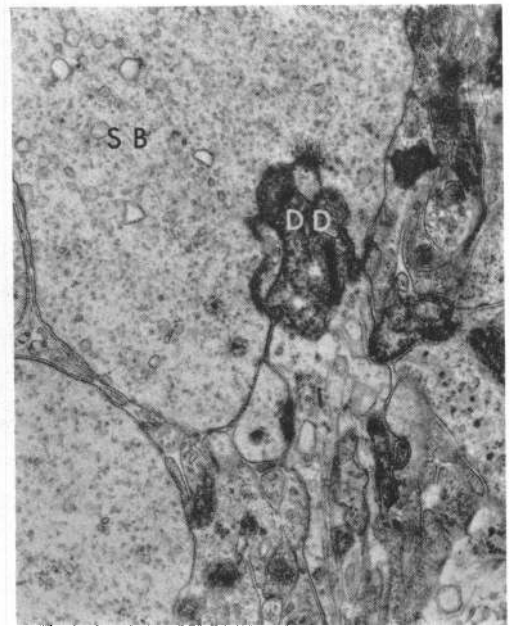

FIG. 36.—Three-day specimen. Outer plexiform layer showing shrunken dense dendrites (DD). SB, synaptic body of visual cell. EM × 14,000.

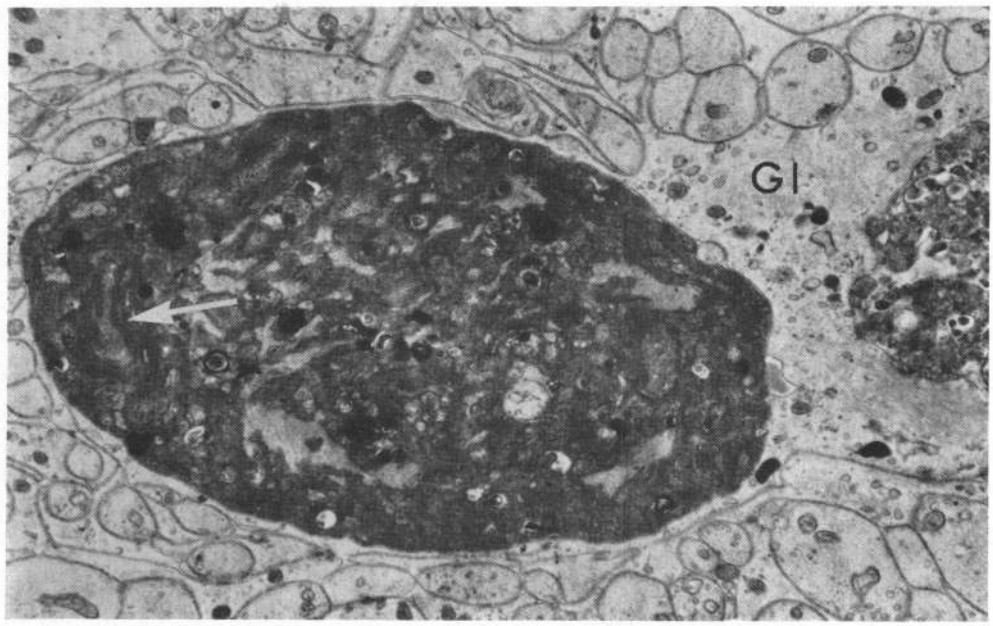

FIG. 37.—Seven-day specimen. Enlarged hypertrophic axon showing a compact mass of highly electron-dense mitochondria, dense bodies, and condensed membranous whorls (arrow). The axon is surrounded by glia (Gl). EM × 8,250.

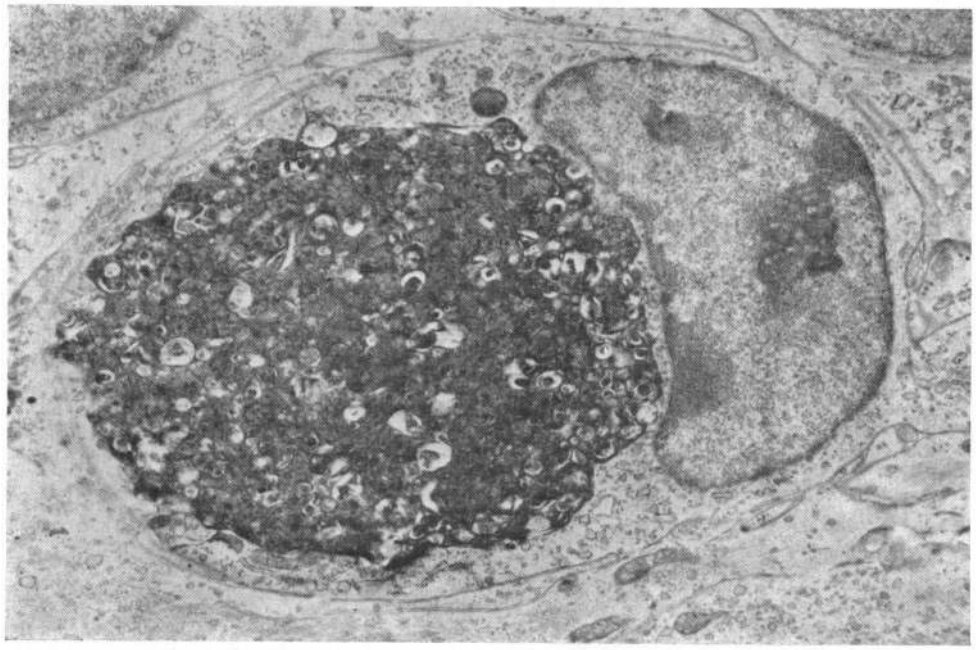

FIG. 38.—Seven-day specimen. A dense and coarsely granular mass from a hypertrophic axon ingested by a macrophage. EM × 9,300.

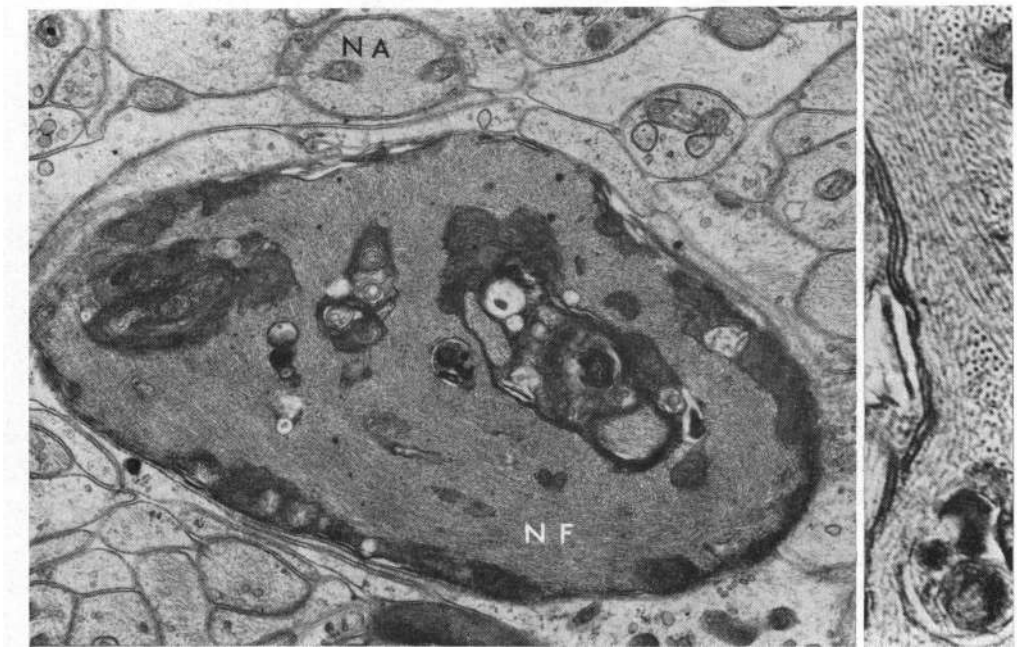

FIG. 39.—Seven-day specimen. Axon showing prominent arrays of interwoven dense, thick neurofilaments (NF) arranged in a finger-print pattern. NA, normal axon. EM · 16,000.
Inset. High power view of neurofilaments. EM · 52,000.

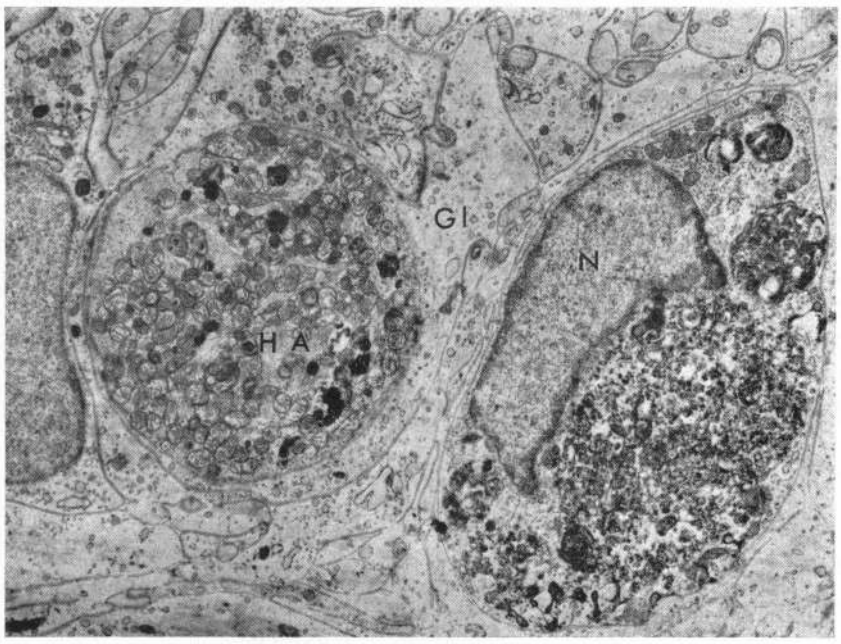

FIG. 40.—Seven-day specimen. Macrophage containing remnants of a hypertrophic axon. Note that the nucleus (N) of the macrophage is pushed towards one side. On the left a hypertrophic axon (HA) is surrounded by glia (GI). EM · 6,600.

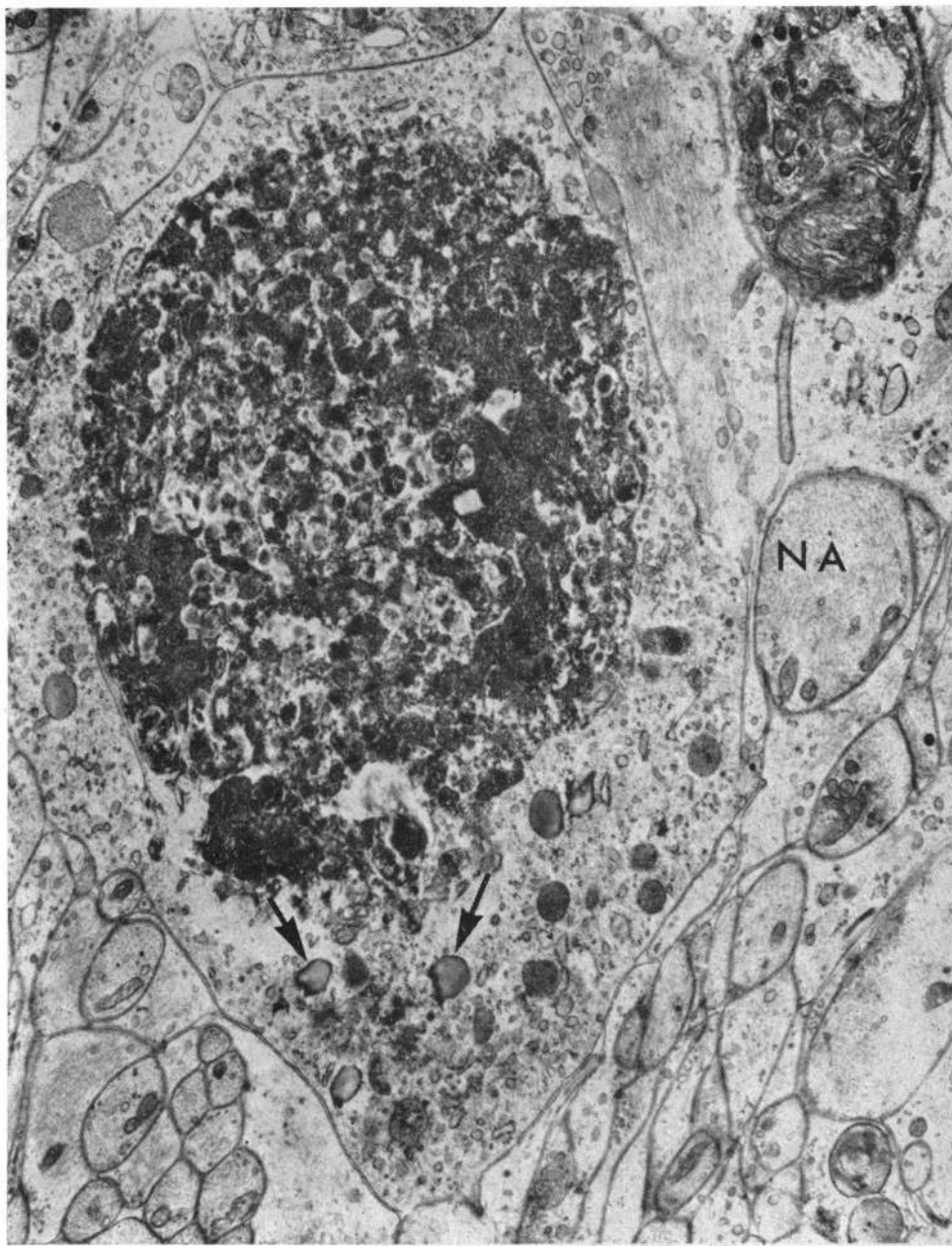

FIG. 41.—Seven-day specimen. Debris from hypertrophic axons ingested by a cell, presumably of astrocytic origin, containing a number of lipid droplets (arrows). NA, normal axon. EM · 13,500.

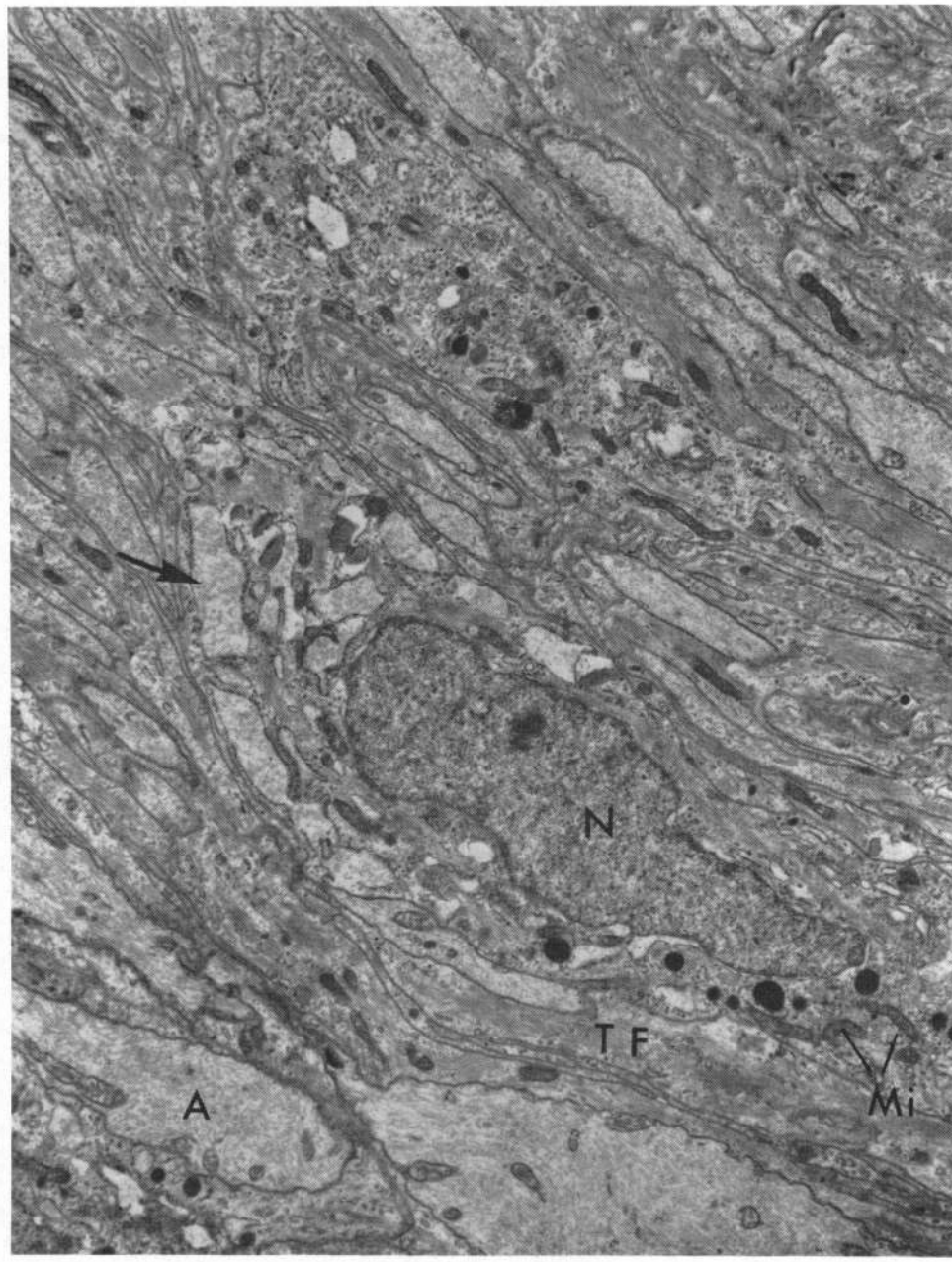

FIG. 42.—Fourteen-day specimen. Occasional axons (A) are seen amongst reactive glia with prominent nuclei (N), dilated endoplasmic reticulum (arrow), dense bodies, lipid droplets, many ribosomes, numerous mitochondria (Mi), and bundles of tonofibrils (TF). EM × 7,500.

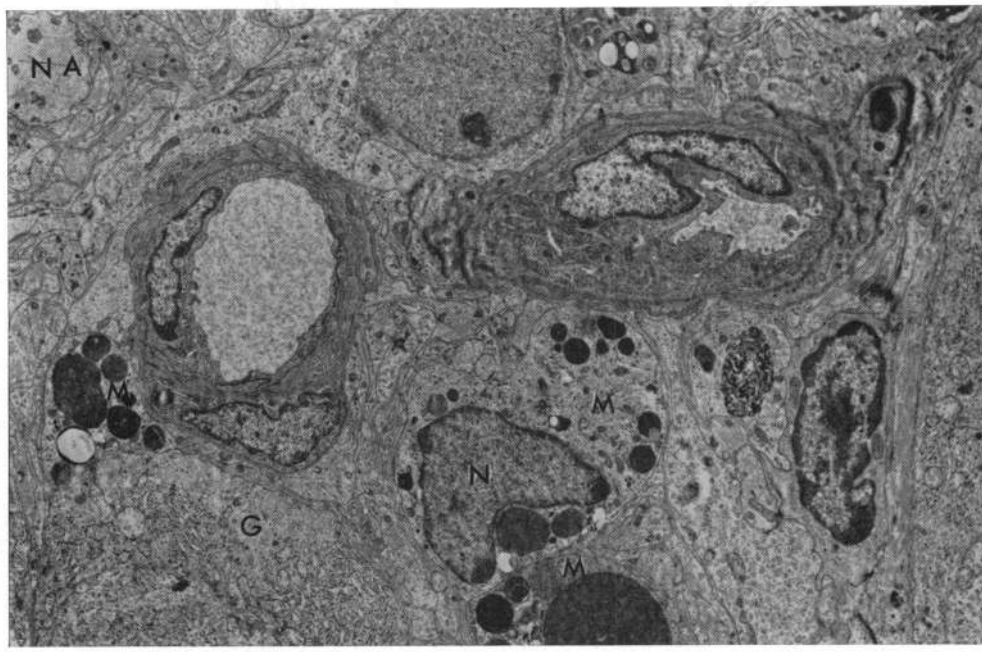

FIG. 43.—Fourteen-day specimen. Macrophages (M) are seen adjacent to blood vessels. One capillary appears open and the other partially closed. NA, normal axon; G, ganglion cell; N, nucleus of the macrophage. EM × 2,800.

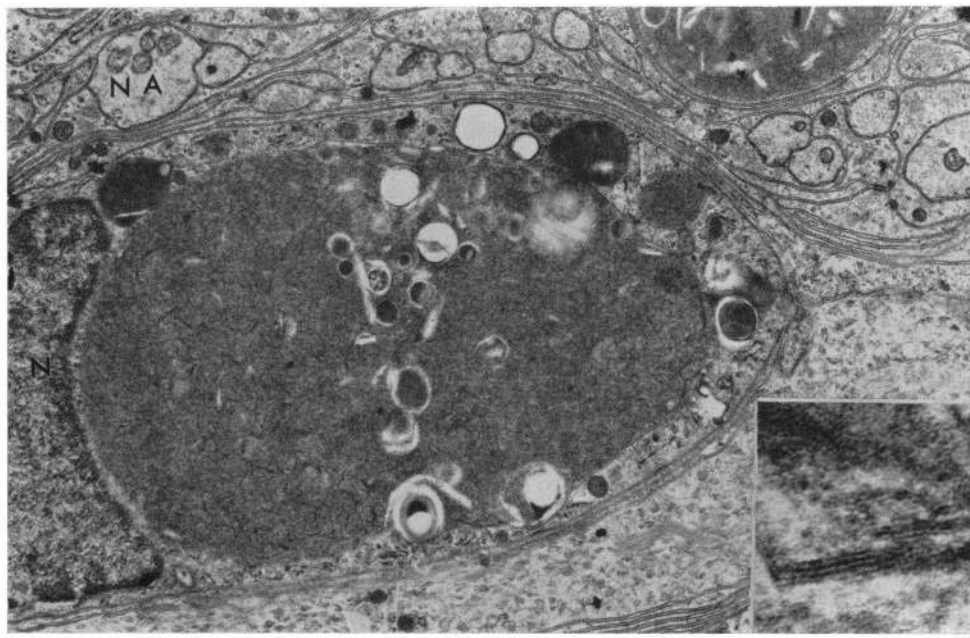

FIG. 44.—Fourteen-day specimen. Remnant of hypertrophied axon appearing as a homogeneous mass, filling the cytoplasm of a macrophage and pushing the nucleus (N) to one side. NA, normal axon. EM × 11,200.

Inset. Higher magnification of the homogeneous mass showing myelin figures. EM × 200,000.

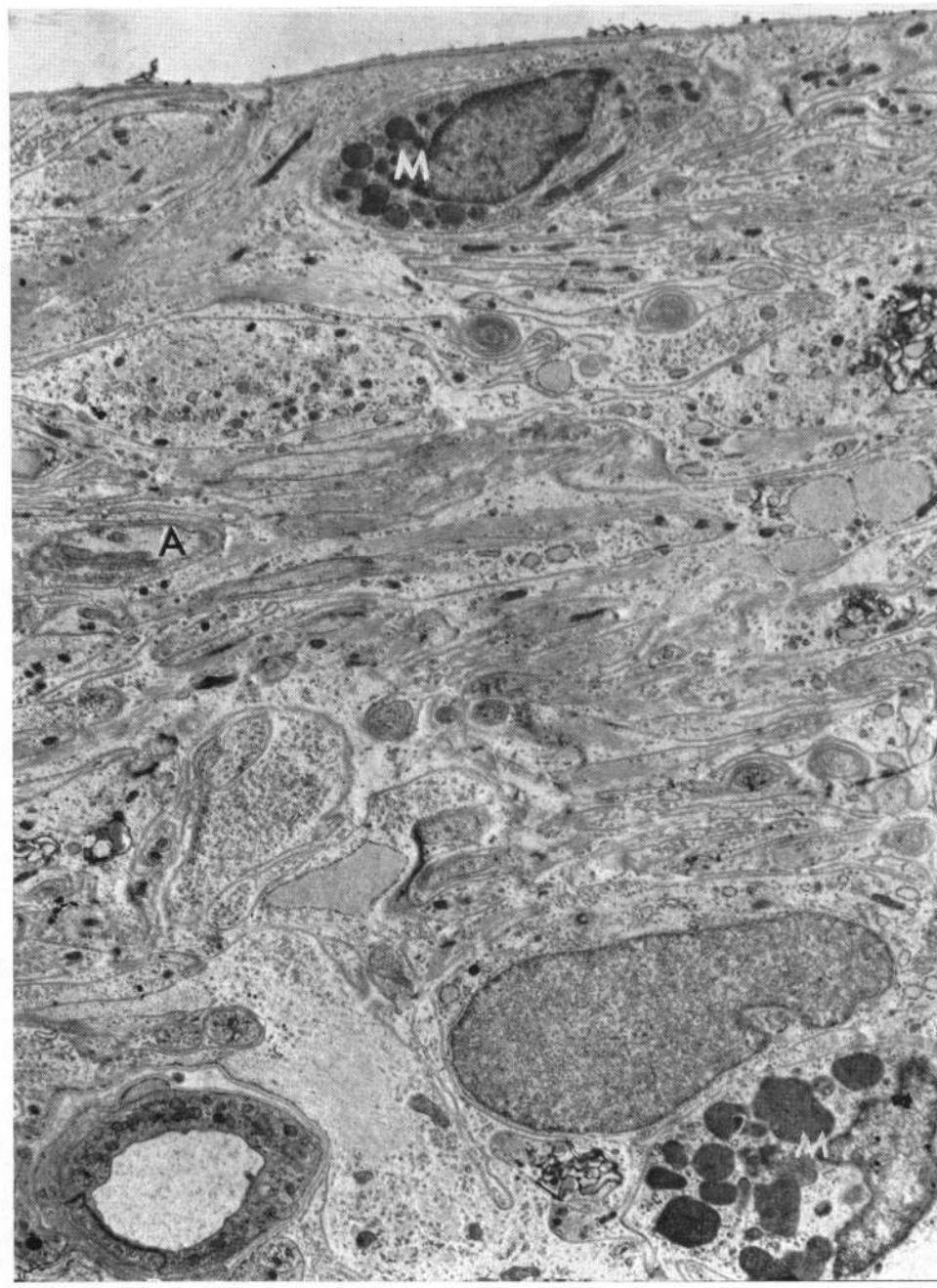

FIG. 45.—Twenty-five-day specimen. Inner retina. Individual layers cannot be differentiated. The compact interwoven glial meshwork contains macrophages (M), blood vessels, and few axons (A). EM × 5,500.

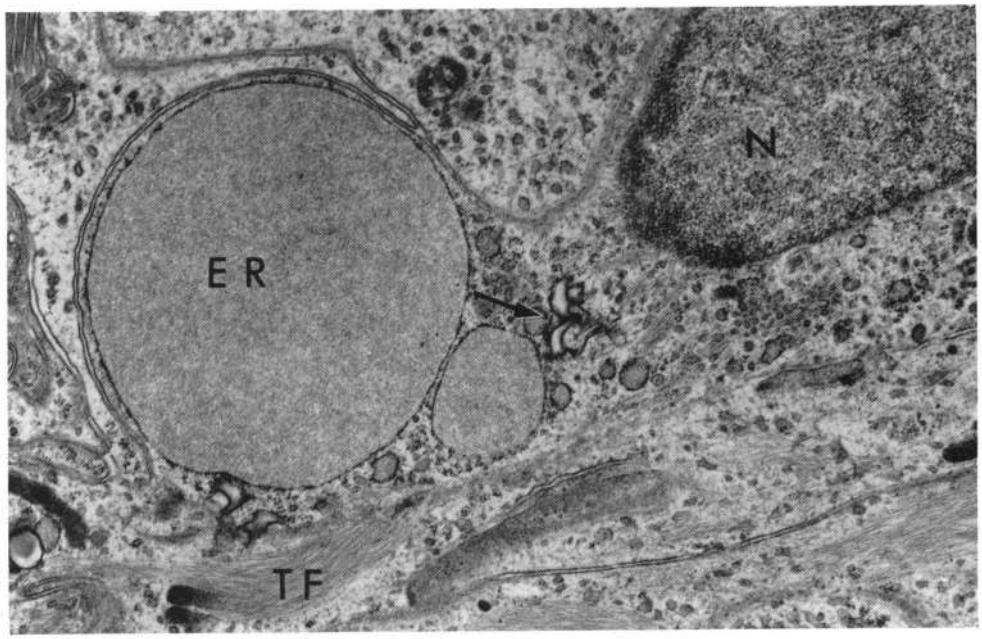

FIG. 46.—Twenty-five-day specimen. Reactive glia containing dense nuclear chromatin (N), compact bundles of tonofibrils (TF), cystic endoplasmic reticulum (ER), lipid vacuoles (arrow), and numerous ribosomes and vesicles. EM × 16,800.

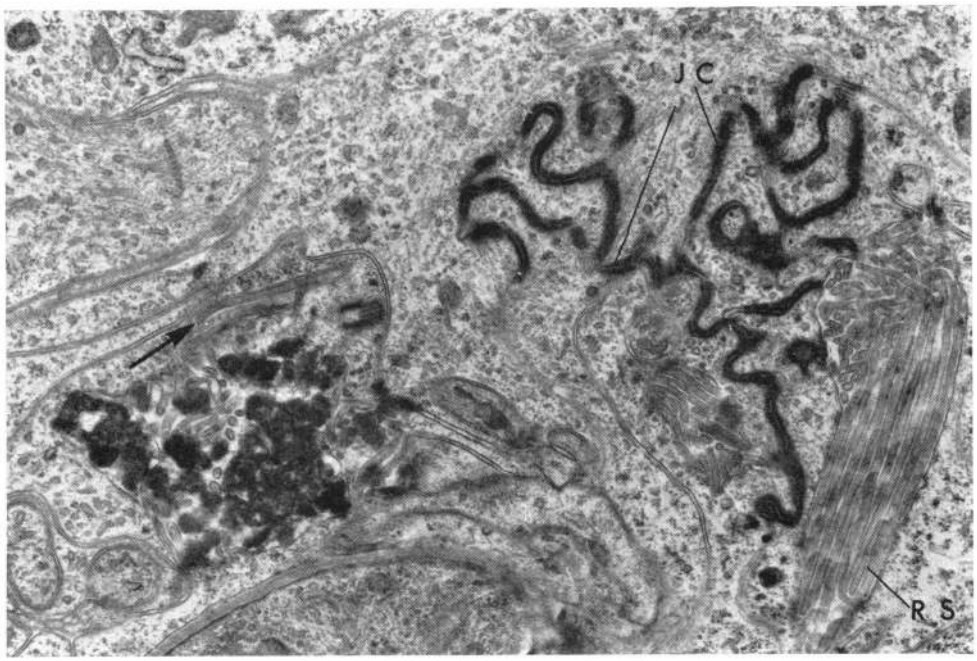

FIG. 47.—Twenty-five-day specimen. Packed rod-like structures (RS), some of which are continuous with the glial cells (arrow), are grouped in the spaces between glia. On the left a few of these are amongst the coarsely granular material. Note the distinct junctional complexes (JC). EM × 14,600.

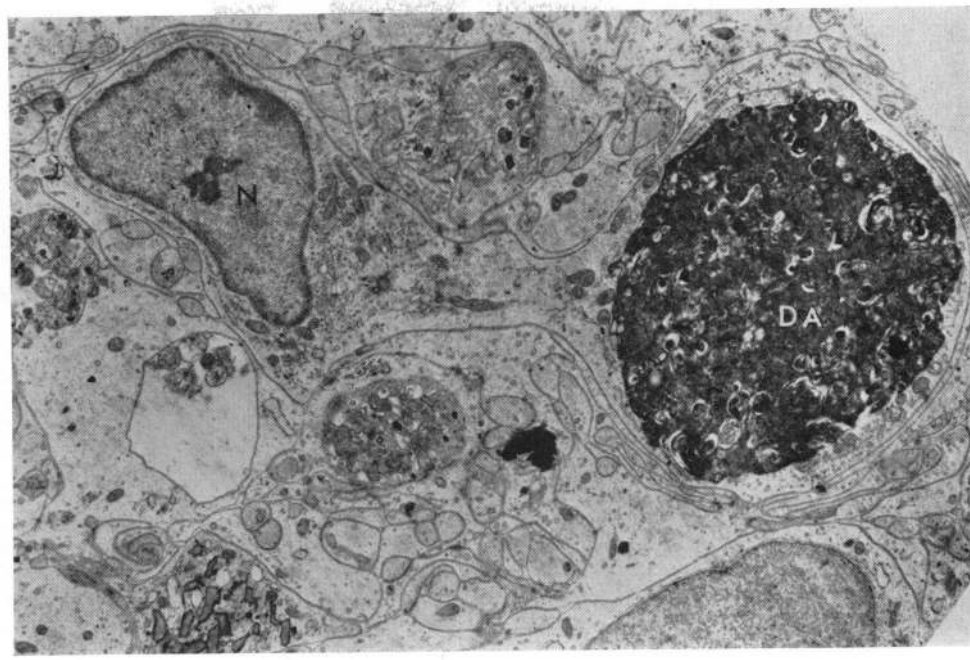

FIG. 48.—A macrophage is seen completely surrounding a degenerated hypertrophic axon (DA). N, nucleus of the macrophage. EM × 6,100.

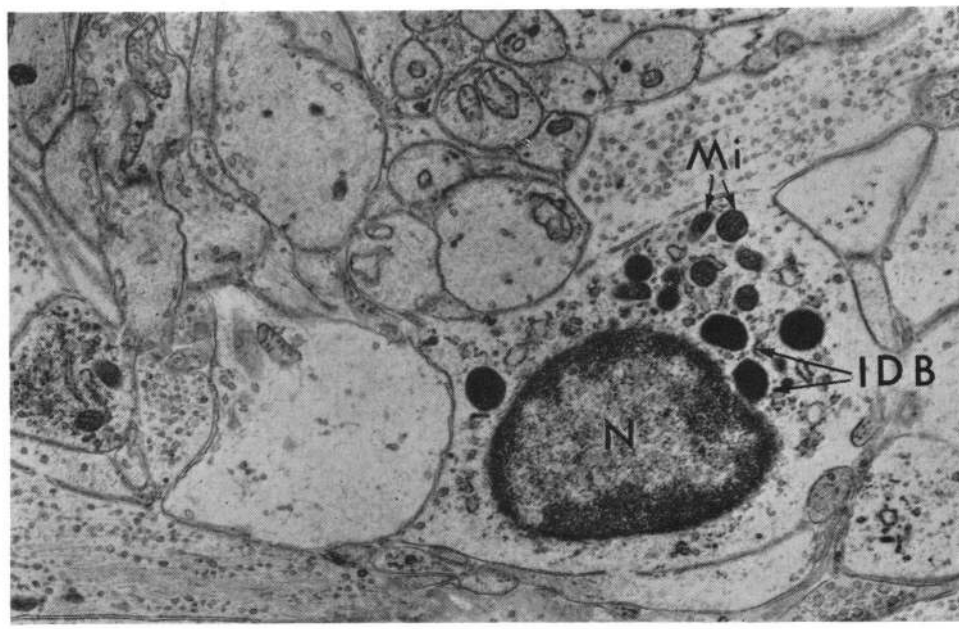

FIG. 49.—A macrophage (histiocyte, microglia) in normal pig retina. N, nucleus; IDB, intracytoplasmic dense body; Mi, mitochondria. EM × 13,000.

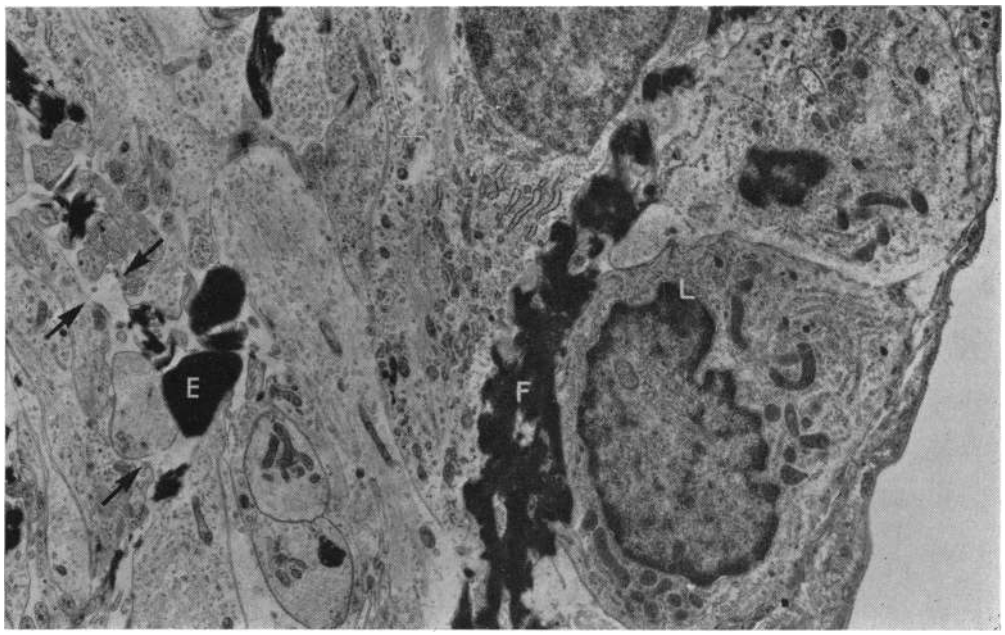

FIG. 50.—Retinal haemorrhage. In the enlarged extracellular space (arrows) erythrocytes (E), leucocytes (L), and filamentous material—fibrin (F)—are present. Note the intact plasma membranes of surrounding retinal tissue. EM × 8,000.

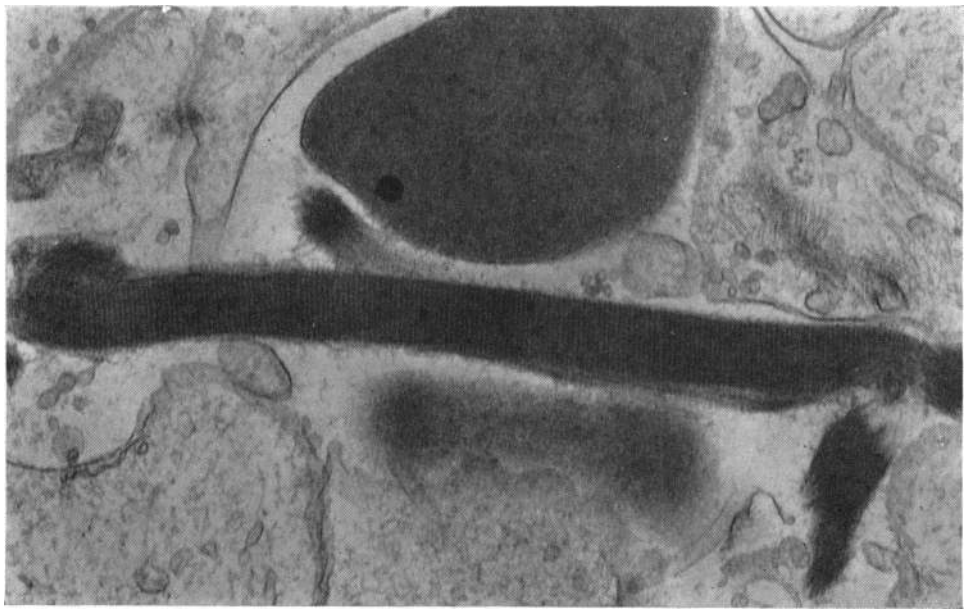

FIG. 51.—Higher magnification of fibrin showing the characteristic banding (150–200 Å). EM × 44,000.

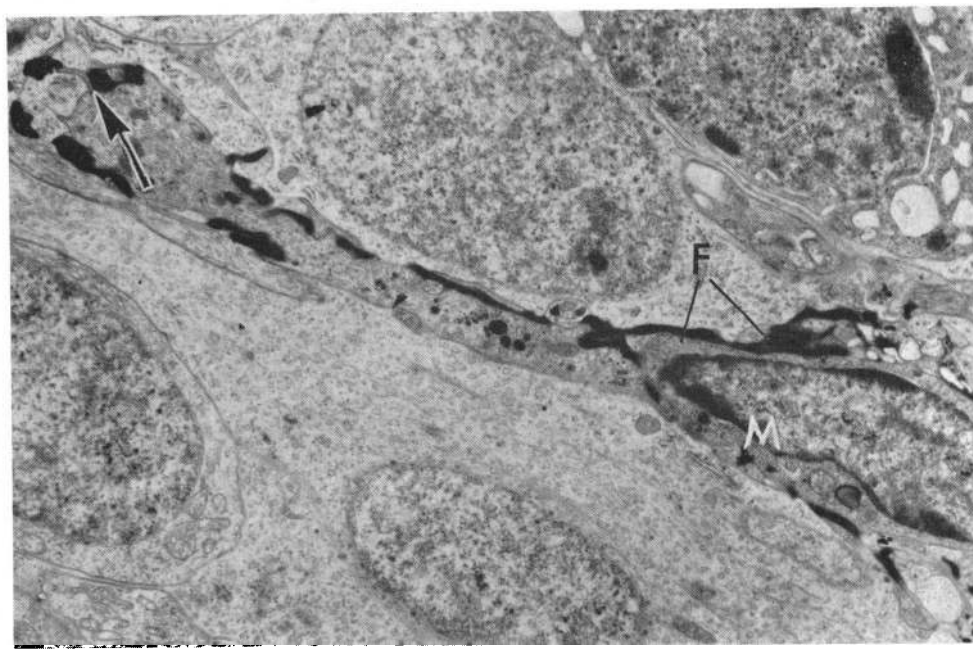

FIG. 52.—Macrophage (M) engulfing fibrin (F). Cytoplasmic projections (arrow) extend towards the fibrin. EM × 4,500.

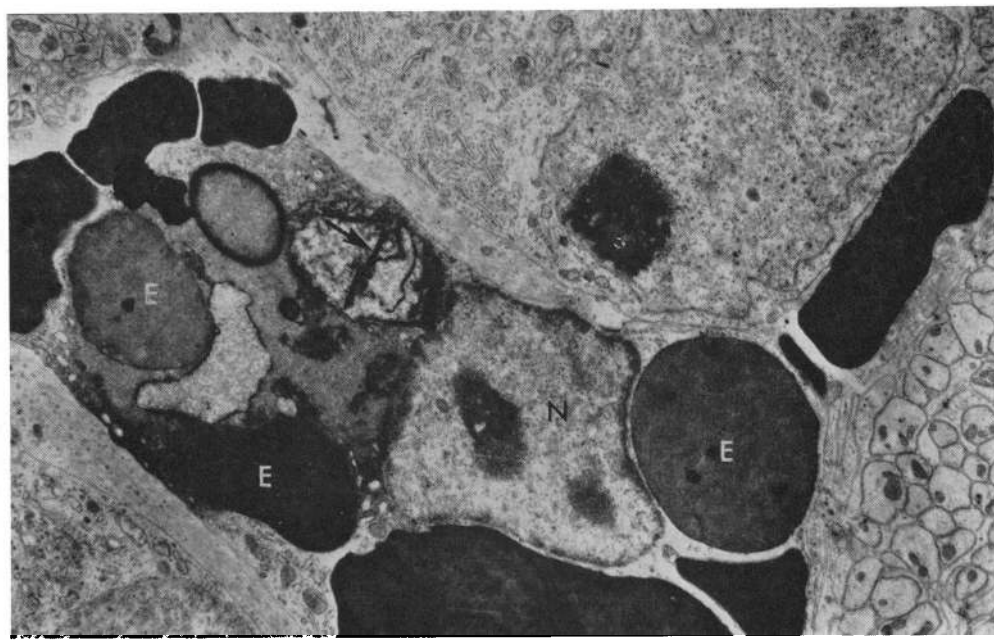

FIG. 53.—Macrophage filled with erythrocytes (E) in different stages of erythrophagocytosis. Note the vacuole containing granular material and electron-opaque bands (arrow). N, nucleus. EM × 8,000.

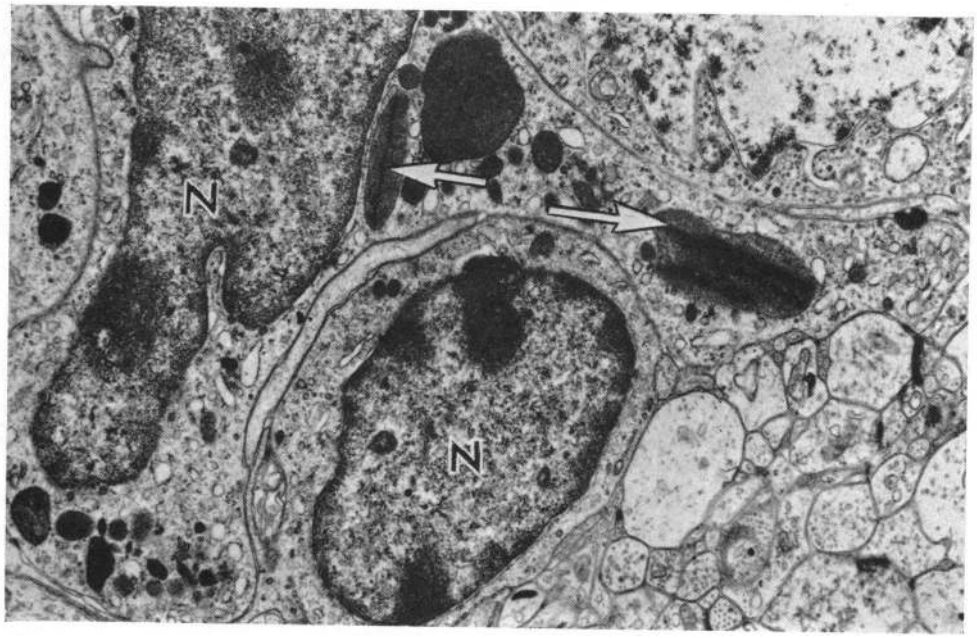

FIG. 54.—Macrophage containing intracytoplasmic granules with electron-opaque bands (arrows). N, nucleus of the macrophage. EM × 10,500.

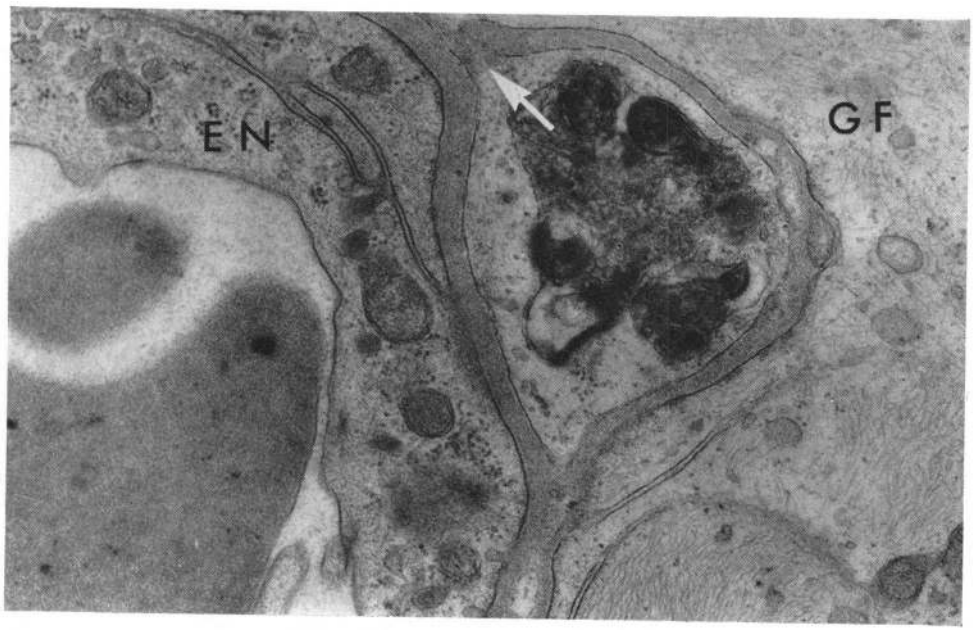

FIG. 55.—Glial fibre (GF) has entered (arrow) the lodge of an intramural pericyte and surrounds the granular debris of the degenerated pericyte. EN, endothelial cell. EM × 36,400.

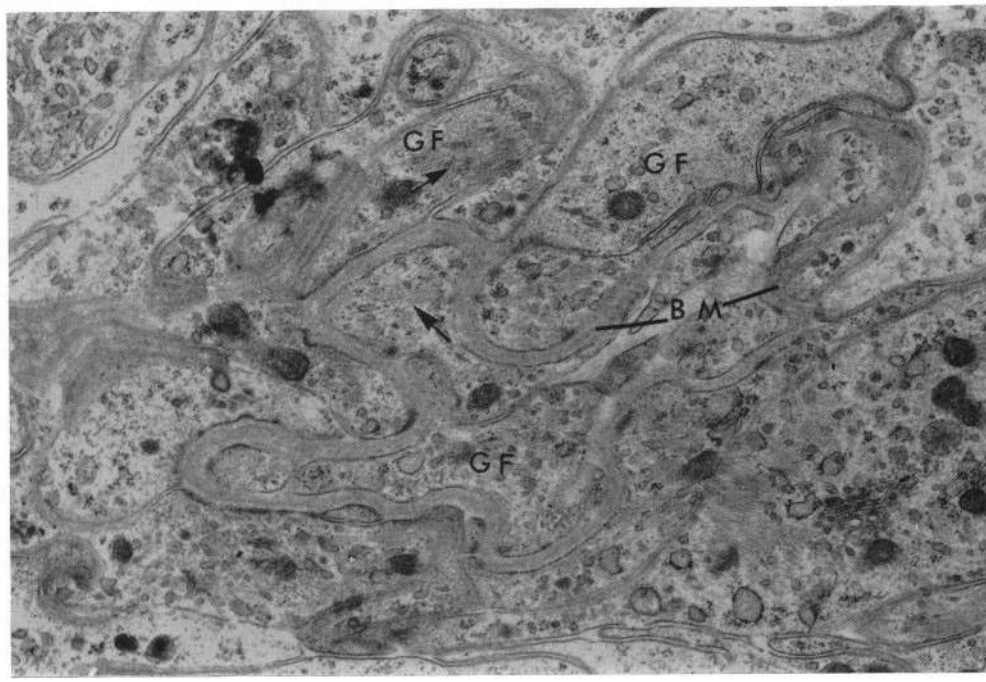

FIG. 56.—Basement membrane material (BM) is surrounded and lined by glial fibres (GF). Note the bundles of tonofibrils in these cells (arrows). EM × 17,600.

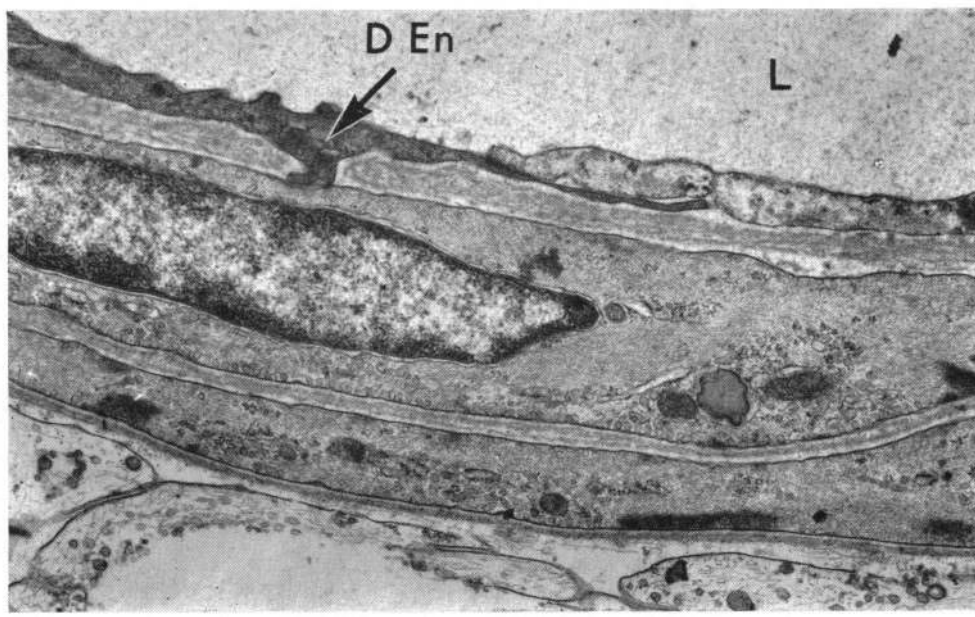

FIG. 57.—Wall of arteriole showing a shrunken and dense endothelial cell (D En). L, lumen. EM × 18,600.

COMBINED SUMMARY

Micro-embolization of the retinal vessels of the pig (using glass spheres) results in the following fundus appearances and corresponding structural changes:

Early Lesions (*up to 5 hours after embolization*)

The immediate result of arteriolar obstruction by emboli was always a great reduction in blood flow to the area of retina downstream. A greyish-white discoloration often developed which corresponded closely with the vascular territory of the blocked vessel. This was not yet a typical cotton-wool spot. Capillary collaterals provided some blood supply to the ischaemic area from the beginning, but this was often ineffective in preventing further evolution of the changes in the inner retinal layers and the formation of a cotton-wool spot. Some areas with good capillary collateral blood supply did not develop cotton-wool spots, particularly when the vessels occluded were small and away from the optic disc.

At an early stage *electron microscopy* revealed simple focal swelling of nerve fibres in the centre of the lesion. Longitudinal sections of some axons showed focal enlargements containing organelles in increased numbers. In the ganglion cells there was depletion of cytoplasmic organelles at the side of the axonal hillock, reduction of ribosomes, swelling of mitochondria and enlarged endoplasmic reticulum with clumping of nuclear chromatin. The inner plexiform layer showed marked swelling and the outer nuclear layer contained swollen cells with almost empty cytoplasm, fragmented cytoplasmic matrix and a few disintegrated mitochondria and vacuoles. It was apparent that the ganglion and bipolar cells were equally sensitive to anoxia, while astrocytes were the most resistant, and Müller fibres occupied an intermediate position.

Fully developed Changes (*24 hours to 4 days after embolization*)

About 24 hours after embolization dense white areas indistinguishable from cotton-wool spots appeared in the ischaemic regions and persisted for about 4 days. Restoration of blood supply to an area after more than 12 hours of ischaemia did not stop the formation of a cotton-wool spot although the blood supply was apparently normal. Once formed, cotton-wool spots cleared during a period of 4 to 14 days, but early return of blood supply did not accelerate the rate of disappearance of the white spot. It appears that the changes in the retina become irreversible after several hours of ischaemia.

Visible cotton-wool spots in the retina were accompanied by characteristic *electron-microscopical* changes with swelling of all cellular elements of the retinal layers and accumulation within the axons of mitochondria, neurofilaments, dense bodies, and inclusion membranous whorls, which were seen especially at the periphery of the lesion, whereas the simple axonal swelling at the centre remained unchanged. Early phagocytosis of degenerated elements was also found.

Late Changes (*4 to 25 days after embolization*)

About 4 days after embolization the typical cotton-wool spot began to fade and became granular in appearance. In light microscopy the ischaemic areas stained darkly with toluidine blue owing to a condensation of the proliferated contents of the swollen axons. Simple swelling had subsided. After about 2–4 weeks macrophages became prominent and this was probably due to their local proliferation. *Electron microscopy* at these stages showed condensed masses of proliferated axonal organelles, ingested within numerous macrophages and reactive astrocytes. Finally the ischaemic area was represented only by a glial scar containing a few persistent nerve fibres, vessels, and phagocytosed material. On the basis of these findings the reactions of phagocytosis, gliosis, and haemorrhage are discussed.

Retinal Vessels

The retinal vessels appear relatively resistant to ischaemia and did not leak or develop aneurysms even when the blood supply suddenly returned through a microsphere becoming dislodged after several hours of obstruction. *Electron microscopically* this relative resistance was confirmed. The endothelial cells and intramural pericytes showed little difference in their vulnerability; it was our impression that, if anything, the pericytes were the more susceptible. In the ischaemic area the lumina of some of the capillaries were narrowed or closed, contained no red cells, and the endothelial cells in these vessels were thickened and concertina-ed as might result from contraction, compression or collapse of the vessel. Endothelial swelling was not seen. The cause of the capillary closure is therefore uncertain.

Dye leakage from the small embolized arterioles did not cause any disturbance of flow through the surrounding capillary bed. Electron microscopy showed that the leaking arterioles had a damaged vascular endothelium.

Cotton-wool Spots

It is concluded that the typical cotton-wool spot is due to intracellular swelling of the inner retinal layers, particularly of the axons, together with proliferation of axonal organelles. It is believed that in whatever clinical condition cotton-wool spots occur the most probable underlying pathogenesis is focal retinal ischaemia from structural or functional arteriolar occlusion. The pseudonucleus of the *cytoid body* almost certainly consists of a conglomeration of proliferating and degenerating axonal structures lying within a pseudocytoplasm formed by the swollen axon itself, or by the cytoplasm of macrophages or astrocytes which have ingested the degenerate material.