AN ELECTRON MICROSCOPIC STUDY OF THE BLOOD-BRAIN BARRIER IN THE RAT, EMPLOYING SILVER NITRATE AS A VITAL STAIN*

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Plates 66 to 70

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The concept of the blood-brain barrier stems from Ehrlich's (1887) observations that subcutaneously injected acid aniline dyes failed to stain the living brain, whereas they freely permeated other tissues. Basic dyes, on the contrary, generally penetrated the central nervous system as readily as other tissues. With the introduction of vital acid–azo dyes, Goldmann (1913) using pyrrhol confirmed Ehrlich's initial observations and gave a detailed account of the hematoencephalic barrier. Subsequent investigators have repeatedly confirmed these observations and have noted that acid dyes and a variety of other compounds bearing negative charges are barred from the central nervous tissue (*cf.* Wislocki and King, 1936; Friedemann, 1942). Trypan blue, which has become the dye of choice for such experiments, does not color the brain of living animals nor can deposits of it be seen in microscopic sections. In the remainder of the body, the tissues are colored a perceptible blue after administration of the dye. Upon microscopic examination, deposits may be identified in macrophages and, in some circumstances, in parenchymatous cells.

The concept of the hematoencephalic barrier provides a generalized explanation for the experimental facts mentioned above. The barrier is assumed to be incompletely developed in fetuses and young animals, since their brains may be stained vitally with trypan blue (Behnsen, 1927; Bakay, 1953). It is absent in regions in which lesions have healed, and it is apparently absent in several circumscribed neural structures including the chorioid plexuses, the neural lobe and infundibulum of the hypophysis, the pineal body, the area postrema, and the intercolumnar tubercle (Wislocki and King, 1936; King, 1938; Wislocki and Leduc, 1952). The last mentioned authors employed a dilute solution of silver nitrate in the drinking water as a vital stain, and noted the close correspondence between the deposits of silver and the deposits of trypan blue. Both substances were absent in the central nervous system with the exception of the

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special regions noted above, which were stained. In these regions, granules of silver or of trypan blue, respectively, were deposited in the walls of vessels and in contiguous cells.

With the development of fixing and sectioning methods suitable for electron microscopy, it has become possible to examine tissues with greater magnifications and resolving powers than heretofore. Since silver is a heavy metal, it seemed likely that silver deposits could be identified by their density in electron micrographs. This proved to be so in an investigation in which dense granules were observed in the basement membranes of liver, kidney, pancreas, and thyroid in animals to which silver had been administered (Dempsey and Wislocki, 1955). We have also identified these granules in tissues fixed in formalin, a fact which obviates confusion with osmiophilic particles. These investigations have been extended to the nervous system, in the hope of delimiting silver deposition more accurately than has been possible formerly. In so doing we have been able to investigate the differences between those regions of the nervous system which do and those which do not bar the passage of vital dyes.

Materials and Methods

Three albino rats and one mouse were used. The animals were maintained in the laboratory animal room on normal diets except that their drinking water contained 1.5 gm. of silver nitrate per liter of drinking water. One rat was killed after 6 months, one after 10 months, and one after 12 months on this régime. The mouse was killed after 8 months. They were killed by decapitation, the brain case opened immediately, and the tissues to be removed were identified by the black deposits of silver contained within them, which contrasted sharply with the unstained contiguous regions of the central nervous system. The neurohypophysis, pineal body, chorioid plexuses of the lateral and fourth ventricles, area postrema, and the intercolumnar tubercle were removed and fixed immediately in Palade's (1952) osmic acid mixture buffered to pH 7.5. The tissues were fixed for 4 hours, washed briefly in distilled water, and dehydrated. They were then infiltrated with a mixture of 3 parts butyl to one part methyl methacrylate and subsequently embedded in this same mixture by catalyzing the monomer with benzoyl peroxide (13 mg./cc.). Polymerization occurred overnight in an oven regulated at 45°C. After hardening, the blocks were sectioned on the microtome described by Dempsey and Lansing (1953). In order to identify the particular regions and to trim the blocks so that the area desired would be obtained, thick sections (1 to 5 μ) were mounted on glass slides and studied by phase microscopy (Houck and Dempsey, 1954). Thin sections (0.01 to 0.03 μ) were mounted on copper mesh grids and examined in a RCA electron microscope model EMU. The specimens were photographed at initial magnifications of 1000 to 7000 diameters, and the negatives so obtained were enlarged photographically as desired.

OBSERVATIONS

Our observations are presented as a series of electron micrographs (Figs. 1 to 9) which display the locations of the deposited silver granules.[1] It appears that

[1] Silver nitrate, an ionic substance, was administered in drinking water to the living animals. Whether the dense granules represent accumulations of reduced silver metal or of silver complexes cannot be stated. It is known that ionic silver combines rapidly with the plasma

the silver deposits occur in homologous locations in all of the regions examined, so that a general account can be used with reference to all of the individual regions.

In regions where silver deposition occurred the heaviest concentrations are invariably encountered in the walls of the small and medium sized blood vessels. Only occasionally were granules visible in the parenchymatous cells. Consequently, the key to understanding the localization of silver and the site of the hematoencephalic barrier lies in the relationships of the morphological elements constituting the walls of blood vessels and their associations with the neural and glial cells.

The capillaries in those portions of the central nervous system in which silver is not deposited, are tubular structures the walls of which are composed of variably flattened endothelial cells partially overlapping one another in an imbricated pattern. The plasma membranes of these endothelial cells are well defined, but extremely thin structures about 100 A in thickness. At the junction of two endothelial cells, the plasma membranes are closely apposed to one another, a space of about 200 A separating the two cells walls. Around the periphery of the capillary, and external to the endothelium, cellular processes containing mitochondria and other cytoplasmic structures may be seen (Fig. 4). These processes apparently are extensions of glial cells, since their cytoplasmic configurations are similar to those seen in glia and in favorably oriented sections, they may be seen as pseudopod-like extensions of glial cells. These processes are closely apposed to the peripheral margins of the capillary endothelial cells. Between the plasma membranes of the glial and endothelial cells, a space can be detected; this space is variable in thickness and appears to be filled by an amorphous ground-substance of moderate electron density. Thus, no fibrils or other connective tissue elements surround the capillary; on the contrary, the endothelium is associated closely with the glial end-feet and is separated from them only by a thin layer of interstitial substance.

Unlike the capillaries described above, those found in the specialized regions selected for the current study (the regions in which silver granules are found) lack such an intimate association with glial end-feet. In these regions, a lamina of variable thickness and with a homogeneous, moderate density is closely

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albumins; nevertheless, this reaction is an equilibrium and over the long periods of time involved, direct reduction from the ionic form could occur. The mechanism involved in this kind of silver transport has been discussed previously (Dempsey and Wislocki, 1955). The problem is of some pertinence in the present investigation since the hematoencephalic barrier is presumably impervious to substances carrying a negative charge only, yet silver, which is a cation, is deposited in much the same locations as are the anionic dyes. It would seem most likely, therefore, that the silver is transported as a complex, probably a proteinate, carrying a net negative charge. In any event, the terms "silver granule" and "silver deposits" are used in the following passages in the sense that they indicate only the presence of silver but are non-committal as to its physical or chemical state.

applied to the outer margins of the endothelial cells. This lamina is similar in appearance to the basement membranes associated with capillaries elsewhere in the body. Like them, it is a prominent site of silver deposition (Figs. 1, 5, and 6; *cf.* Dempsey and Wislocki, 1955). Surrounding this capillary basement membrane is a region in which typical connective tissue elements may be seen. Collagenous fibrils, either solitary or occurring in bundles, are invariably present. Occasional cells are encountered which with flattened nuclei, a moderate amount of ergastoplasm, and wavy, indistinct cell margins present the typical appearance of fibroblasts. Surrounding this region with its cells and fibers, and delimiting its outer margins, is another amorphous basement membrane upon which the glial, neural, or glandular elements rest. Like the capillary basement membranes, this one too contains many silver granules. Between the capillary and parenchymal basement membranes, branches or extensions of one or the other frequently occur. These extensions often surround bundles of collagenous fibrils.

The foregoing general account of the relationships of the blood vessels and the nervous tissue describes the principal differences between the specialized regions in which silver deposition occurs and those which bar silver nitrate. With these considerations in mind, we shall now present descriptions of each of the silver-storing locations.

Chorioid Plexus.—The disposition of the various elements of the chorioid plexus follows closely the general pattern described above. The cuboidal cells rest basally upon a definite membrane in which granules of silver are located (Fig. 1). Beneath this membrane fibroblasts and collagenous fibrils are visible. Here and there, but particularly in regions where indentations occur in the basement membrane of the chorioidal epithelium, the subjacent fibroblasts have been seen to exhibit complex and intertwining extensions of their cytoplasm to form tangled knots. In the interstices between these processes, and on their surfaces, silver granules are frequently encountered. Bundles of collagenous fibrils are often associated with these knot-like structures; in places the fiber bundle is completely surrounded by the cellular projections (Fig. 1, arrows). Adjacent to the connective tissue space with its cells and fibers is another basement membrane associated with the capillary wall. Like the membrane of the epithelium, this one also exhibits silver deposits.

Arterioles and small arteries supplying the capillaries of the chorioid plexus also exhibit deposits of silver in their walls. A basement membrane underlies the arteriolar endothelium exactly as in the case of the capillary. External to this are the smooth muscle cells comprising the muscular media of the vessel. Surrounding each individual muscle cell is an amorphous, moderately dense membrane. This investment of the muscle fibers forms continuous anastomosing connections with the endothelial basement membrane, and contains silver deposits embedded within its substance (Fig. 2). Collagenous fibrils are inti-

mately associated with all of these basement membranes. In favorable locations fibrils with their characteristic cross-striations can be observed running toward the amorphous membranes and disappearing into their substance (not illustrated, but compare Fig. 3).

The chorioidal epithelium contains numerous rather small, spheroidal or elongated mitochondria. Numerous vacuoles more or less circular in section and bounded by a dense, granular outer membrane are encountered. Interspersed between these elements large numbers of smaller bodies make up the ground-substance of the cytoplasm; in low-power micrographs these appear as dots, but at higher magnification they become resolved into circular objects about 500 A in diameter and exhibit a clear center surrounded by a smooth membrane. In occasional cells there are vacuoles containing silver particles dispersed in a foamy substance. At the apical or free surface of the cells, the plasma membrane may appear as a smooth surface, but more often it is thrown into folds covering bulbous protoplasmic projections.

Area Postrema and Intercolumnar Tubercle.—In the micrographs, the relationships of the vessels to the neighboring tissue and the location of silver in the various elements were identical in these two regions. The regions will, therefore, be described together. Both regions are commonly described as containing vascular sinuses and glial cells (King, 1937; Wislocki and Leduc, 1952). Nerve cells, sometimes pigmented, are occasionally present in the human area postrema (Cammermeyer, 1944). In animals fed silver nitrate for long periods, the deposition of silver is heaviest in the walls of the vessels nearest to the surface of the ventricles (*cf.* Wislocki and Leduc, 1952). In the more deeply situated vessels the deposition of silver is less so that there is no sharp line separating the area postrema or the intercolumnar tubercle, respectively, from the underlying regions possessing a well defined hematoencephalic barrier. It is possible, therefore, to observe fields in adjacent regions in which heavy deposits of silver are present, others in which only slight amounts can be seen, and still others with none.

In the superficial vessels with heavy deposits silver occurs in a well defined endothelial basement membrane, among the collagenous fibrils of the connective tissue space surrounding the capillary, and in an outer lamina of amorphous substance which follows a tortuous course separating the glial tissue from the perivascular connective tissue. In the most superficial regions, the outer lamina seems to be complete and the outer limits of the connective tissue exhibit relatively smooth contours (Fig. 5). Somewhat deeper, glial processes interdigitate extensively with the connective tissue, but the two are always separated by the membrane containing silver granules and at still deeper regions the outer membrane apparently becomes incomplete. Here and there, glial processes may be seen penetrating as far as the endothelial basement membrane. The outer membrane, in such locations, can sometimes be followed

as it wanders off into the surrounding tissue between the glial processes. Finally, in the deepest regions the outer membrane disappears, the capillaries lose their basement membranes as well, and no silver is found.

Occasional vacuoles containing silver have been observed in the fibroblasts of the perivascular connective tissue space and in the glial cells (Fig. 7).

Neurohypophysis.—The capillary vessels of the posterior pituitary gland are surrounded by perivascular connective tissue which is bounded internally by an endothelial basement membrane and externally by an outer membrane. Between these argyrophilic membranes connective tissue cells and fibers occur in a disposition entirely similar to the depositions described above for the other regions in which silver deposits are found. Frequently, but by no means invariably, silver deposits may be seen in occasional amorphous membranes which run between the pituicytes and their processes. These latter elements may be identified by their locations and by the presence within their cytoplasm of osmiophilic lipide droplets which have been seen in tissues from control animals (Fig. 9). The membranes and silver granules associated with them seem to pursue indiscriminate courses through the gland. They exhibit occasional branches, and in favorable locations they may be seen to connect with the outer membrane of the perivascular connective tissue.

Pineal Body.—Capillaries in the pineal body are surrounded by connective tissue investments similar to those described in the preceding section for the neurohypophysis. Silver deposits occur in the endothelial basement membrane (Fig. 8), scattered among collagenous fibrils, and in the outer membrane from which strands and branches fray off to run in irregular courses between the parenchymal cells. Occasional intracellular deposits are encountered in vacuoles located in the pericapillary fibroblasts and, less frequently, in the pineal cells.

DISCUSSION

The observations presented above, involving the location of silver deposits in nervous tissues from animals to which silver nitrate had been chronically administered, throw light upon the elements responsible for the hematoencephalic barrier. This barrier constitutes a mechanism which prevents various negatively charged substances, including vital dyes and presumably chemically similar metabolites, from entering the nervous tissue from the blood stream. Evidence is at hand indicating this barrier is a partial one. Thus Behnsen (1927, page 569) found, upon administering trypan blue to newborn and young mice, that the dye was deposited widely, although for the most part faintly, in the brain and spinal cord. With age, he observed that both the extent of the staining and its intensity diminished greatly, so that in the adult only a few regions (neurohypophysis, pineal body, chorioid plexuses, etc.) persisted which stored the dye with undiminished intensity. Similarly, Bakay (1953) observed the existence of a blood-brain barrier for P^{32}, which was more permeable in fetal and young rabbits than in adults. Experimental damage to the brain

modifies or destroys the barrier, so that vital dyes can accumulate in regions where lesions have healed (Macklin and Macklin, 1920).

Besides the small special areas of the brain (neurohypophysis, area postrema, intercolumnar tubercle, and pineal body) which are permeable to vital dyes and silver, Wells and Carmichael (1930) encountered vitally stained "microglia" throughout the periventricular gray matter of rabbits. Wislocki and Leduc (1952, 1954) reported a similar deposition of silver within "glial" cells of the periventricular gray substance of rats, especially abundant along the cerebral aqueduct and on the outskirts of the area postrema and intercolumnar tubercle. Moreover, these cells which stored exogenous substances, normally contained a proteinaceous material which was selectively stained by chrom alum–hematoxylin (Wislocki and Leduc, 1954). Neural cells which stain vitally and store silver have also been noticed in some abundance in the neurohypophysis and to a lesser extent in the area postrema and intercolumnar tubercle (Wislocki and King, 1936; Wislocki and Leduc, 1952). The reactive cells of the infundibular process were considered to be pituicytes. In view of these findings, it is not surprising that, in these regions, the electron microscope has revealed various cells containing granules of silver.

In addition to the cells referred to above, silver is deposited in large amounts in the connective tissue sheaths of the peculiar sinusoidal capillaries which are characteristic of the neurohypophysis, area postrema, intercolumnar tubercle, and pineal body. These deposits are conspicuous, not only in the light microscope (Wislocki and Leduc, 1952), but also in the electron microscope as shown in the present investigation. Silver is similarly encountered in the connective tissues sheaths enclosing the wide calibered capillaries of the chorioid plexuses. Indeed, from our observations, it appears that wherever in the brain capillaries are associated with an adventitial coat of connective tissue, silver storage occurs. In a previous study we have observed silver deposits in the basement membranes surrounding capillaries of the pancreas, thyroid gland, and kidney, as well as in segregation vacuoles in macrophages and fibroblasts (Dempsey and Wislocki, 1955). Similarly, in the present investigation, we have observed connective tissue cells, membranes, and fibers disposed around the capillaries of the area postrema, intercolumnar tubercle, pineal body, neurohypophysis, and chorioid plexuses. In all of these regions, silver deposition has occurred in the connective tissue elements. On the other hand, the capillaries of other regions of the nervous system which we have examined, including the spinal cord (Fig. 4), internal ear, cerebellar cortex, and the reticular formation of the third and fourth ventricles, lack any investment of connective tissue elements, and do not store silver except for the occasional deposits in glial cells mentioned above and illustrated in Fig. 7. These results prompt the suggestion that where connective tissue surrounds the capillary endothelium, silver storage occurs, but where the endothelium rests on an abutment of glia, silver is barred.

The foregoing descriptions raise questions concerning the possible presence

of an interstitial ground-substance of the nervous system. Hess (1953) has recently reviewed the evidence for such an interstitial material, and has shown that sections of brain exhibit a faint positive reaction with the periodic acid–Schiff procedure. This reaction is not abolished by treatment of the tissue with solvents which normally remove the connective tissue mucins. Moreover, a slightly stronger reaction occurred in a zone surrounding small blood vessels including those of capillary diameter. In our present photographs, the elements constituting the glial processes, endothelium, and nerve fibers are so closely applied to one another that one is at first tempted to deny the occurrence of an interstitial substance on the ground that there is no room for it. However, close examination of the neuropil in electron micrographs reveals that the plasma membranes of adjacent glial processes are indeed separated from one another by a space, narrow through it may be, and that this space contains an amorphous substance of moderate electron density. This substance is continuous with that underlying the capillary endothelium and separating it from the glial end-feet (Fig. 4). The amount of this amorphous ground-substance is variable, both in its location between glial elements and in its position as a capillary basement membrane. In most parts of the central nervous system silver deposits do not occur in this ground-substance, but in the pineal body and neurohypophysis there are regions in which the outer membrane of the perivascular connective tissue appears to be continuous with it and in such locations silver granules may be seen in the spaces between glial processes. From all of these observations, we suggest that a thin lamina of interstitial material occurs between the neural cells, that this substance differs from the connective tissue ground-substance in that it does not attract silver deposits, and that it is miscible with the connective tissue ground-substance in at least some locations. Whether this neural ground-substance acts as the hemato-encephalic barrier or whether the glial plasma membrane is the region which bars silver cannot be determined from our observations.

Both in the central nervous system and in other parts of the body, mechanisms apparently exist for barring silver from parenchymal cells. The mechanisms are apparently different, however, depending upon whether blood vessels have a glial investment or a connective tissue sheath. In the brain, silver does not accumulate readily nor in great amounts in the tissue, apparently because it cannot escape from blood vessels. The presence of an impenetrable barrier could account for the fact that no silver is deposited in the brain. On the other hand, where connective tissue investments occur, silver clearly escapes from the blood stream since it is deposited copiously outside the endothelial cells. Here, however, it is segregated by macrophages, ground-substance, and basement membranes. These structures presumably act as filters, removing the silver from the plasma filtrates before they reach the parenchymal cells. In the brain, therefore, silver is turned back into the blood plasma whereas in the

rest of the body it is adsorbed out of the tissue fluids before they reach the parenchymal cells. In both cases, the parenchymal cells are protected from exposure to poisonous concentrations of the toxic heavy metal. Silver reaches the parenchyma only when these protective devices are presumably overwhelmed by too high a concentration of silver, or when a pathological breakdown of the barrier occurs.

In a previous investigation (Dempsey and Wislocki, 1955) we have called attention to the segregation of silver into occasional vacuolar structures found in the parenchymal cells of the liver, kidney, and pancreas. These structures were similar in size, shape, and location to the mitochondria of these organs. Furthermore, in vacuoles with relatively small amounts of silver, internal folds like those of mitochondria were present, and the outer walls of the vacuoles were lined by a double membrane, the dimensions of which were comparable to those of mitochondria. These observations prompted us to suggest that the silver segregation vacuoles were derived from mitochondria, and to speculate that such a pathological change in mitochondria might account for the toxicity of silver salts since mitochondria are now known to contain many enzyme systems necessary for the normal activity of cells. Our present findings do not illuminate this problem further, although no evidence contraindicating such a possibility has been forthcoming. Occasional silver-containing structures have been seen in the glial cell processes, and in the cells constituting the parenchyma of the chorioid plexuses, pineal body, and posterior pituitary gland. These vacuoles are of a size and shape such that they could have been derived from mitochondria. However, we have only rarely been able to observe any with internal folds characteristic of mitochondria (Figs. 5 and 7) and so their origin remains problematical.

It should also be mentioned that in none of our observations on tissues from animals to which silver nitrate had been administered, has silver ever been encountered in endothelial cells. This is surprising since the silver must be transmitted through the endothelium before it can be stored in the connective tissues, basement membranes, or parenchymal cells. Because of our suspicion that silver exerts its toxicity by damaging mitochondria, we have examined these bodies in endothelial cells with especial care. We have been struck by the fact that mitochondria are relatively rare in endothelial cells, and especially that they are practically absent in the thin portions of the cells. The few that are present congregate near the nuclear region and in nodal thickenings. It is possible, therefore, that silver is normally transmitted through the endothelium in regions where mitochondria are absent, and that these elements are, therefore, relatively immune to its toxic effects. It is also possible that the endothelium has some mechanism for the transmission of fluid and solutions without their intermingling with the cytoplasm of the cells. Such a mechanism has been suggested by Palade (1953) who described small vacuoles which were

postulated to form on the luminal side of the endothelium, migrate across the membrane, and discharge their fluid contents at its basal margin.

Our observations throw some light on the problem of the so called perivascular, or Virchow-Robin, spaces. In the leptomeninges where large vessels turn inward into the brain substance, the subarachnoid space is also reflected inward along with the vessels to form cylindrical perivascular extensions. Weed (1922, 1923) injected the Prussian blue reagents into the subarachnoid space and was able to identify the pigment in the perivascular cuffs of the larger penetrating vessels. Prussian blue was not found along the walls of the smaller vessels, however, unless the animal was exsanguinated or the blood rendered hypertonic by sodium chloride. Weed, therefore, postulated that the Virchow-Robin spaces, ordinarily seen as histological spaces separating the endothelial cells from the glial processes, carried fluid from the capillaries toward the subarachnoid reservoir, and that only under conditions as extreme as those involving exsanguination or hemal concentration was the direction of flow reversed. It now appears that the Virchow-Robin spaces must be regarded as shrinkage artefacts. Our observations show that the glial processes are separated from the base of the endothelium by only a submicroscopic distance and that between the glia and endothelium is an amorphous substance of appreciable electron density. The Virchow-Robin spaces, therefore, must be regarded as artificial separations caused by shrinkage of the glial processes away from the base of the endothelium. The regularity with which shrinkage occurs at this location, and the fact that the Prussian blue reagents diffuse along a perivascular route after exsanguination, indicate that the interstitial ground-substance, if any, is tenuous and easily distorted by mechanical stresses.

SUMMARY AND CONCLUSIONS

The intravital deposition of silver in the chorioid plexuses, area postrema, intercolumnar tubercle, neurohypophysis, and pineal body of rats, given 1.5 gm. of silver nitrate per liter of drinking water for periods of up to one year, has been investigated by electron microscopy. Unlike other parts of the central nervous system, these regions store large amounts of silver. In all of these structures, silver is deposited in the form of dense granules in the basement membrane upon which the capillary endothelium rests, in and upon the connective tissue cells and fibers constituting a loose pericapillary sheath, and in an outer membrane separating this sheath from the parenchymatous cells. Parts of the central nervous system which do not store silver, for example the spinal cord, cerebellar cortex, cerebral cortex, and reticular formation, lack a connective tissue investment of the capillaries. In these locations, the glial processes or end-feet are closely applied to the walls of the capillaries. Only a narrow space, filled by an amorphous, moderately electron-dense substance, separates the plasma membranes of the endothelial cells and glial processes.

The significance of these observations is discussed with respect to the questions of the Virchow-Robin perivascular spaces, the interstitial ground-substance of the brain, and the location of the hematoencephalic barrier.

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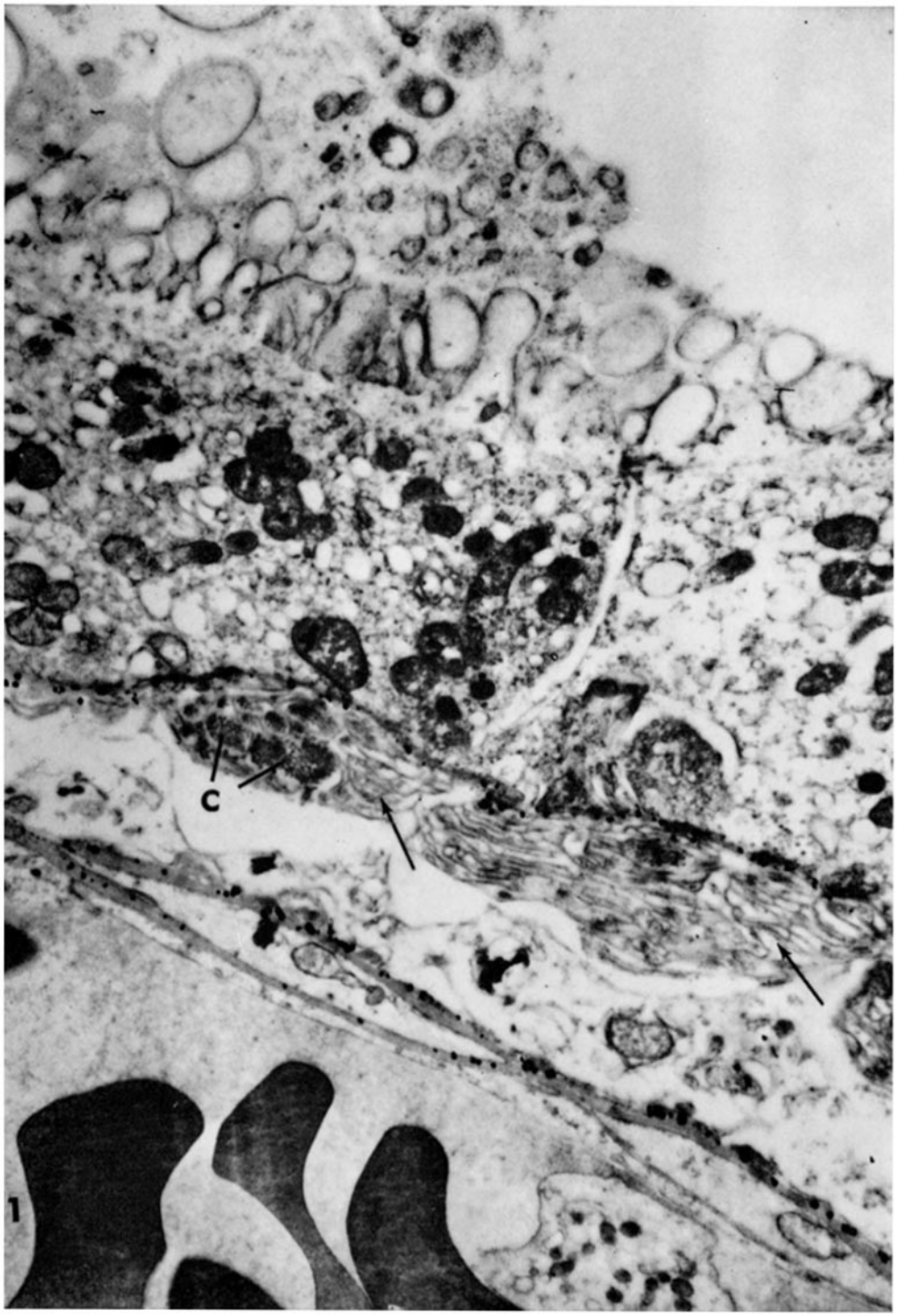
EXPLANATION OF PLATES

PLATE 66

FIG. 1. Section through the epithelium of the chorioid plexus of a rat which had received silver nitrate (1.5 gm. per liter) in its drinking water for 12 months. The lateral ventricle occupies the top right corner, and a capillary containing erythrocytes runs across the lower left of the figure. The chorioidal epithelium rests upon a well defined membrane in which silver particles are located. A similar membrane, also with silver deposits, supports the capillary endothelium. Between these two membranes lies loose connective tissue containing bundles of collagenous fibrils (*C*) and curious knot-like extensions of the plasma membranes of fibroblasts (arrows). The surface of the chorioidal epithelium is irregular—rather coarse pseudopod-like extensions are visible on the left of the figure, whereas along the right margin the surface is much smoother. × 10,000.

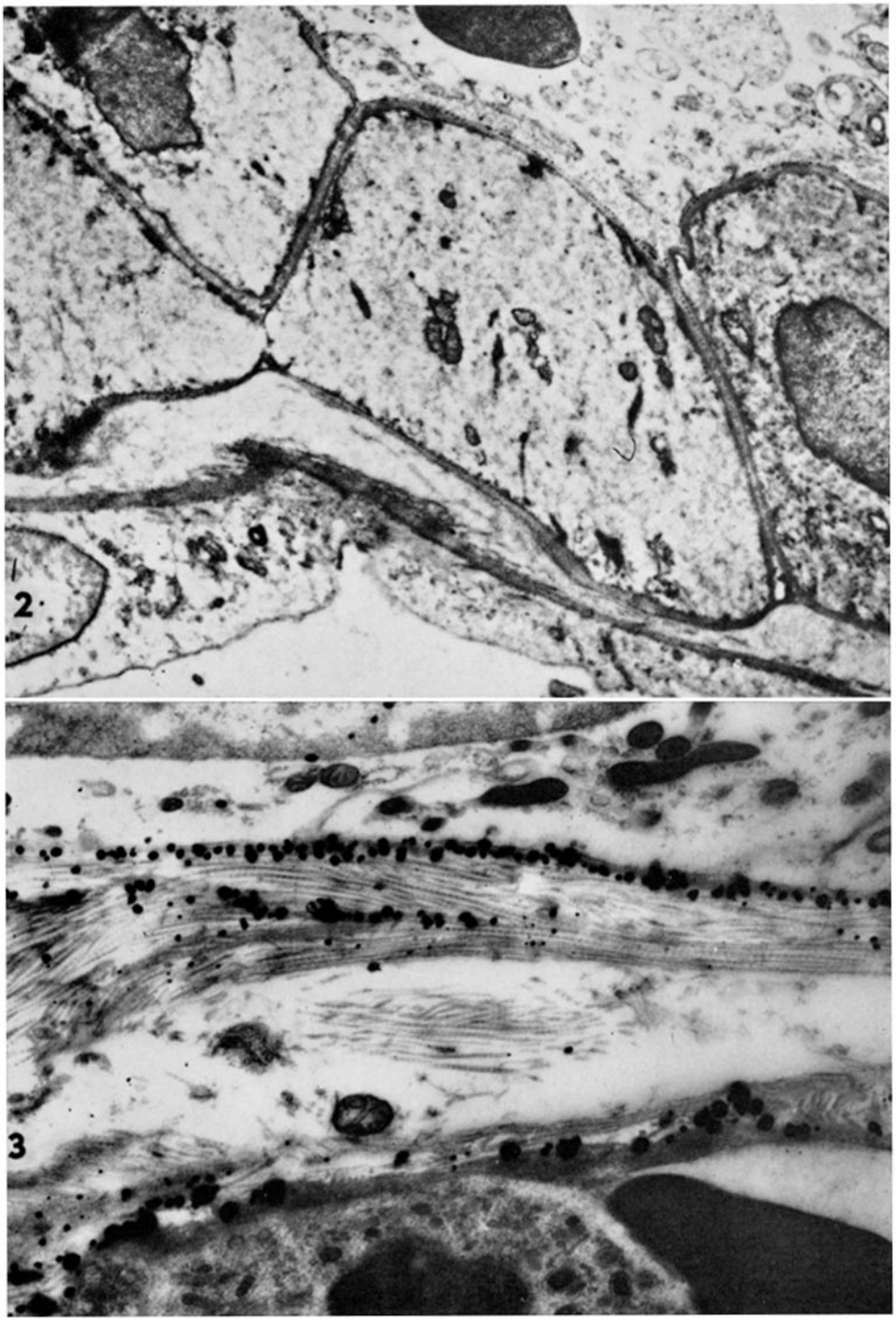


(Dempsey and Wislocki: Electron microscopy of blood-brain barrier)

PLATE 67

FIG. 2. Section through the wall of an arteriole from the chorioid plexus of a rat which had received silver nitrate for 12 months. A structureless sarcolemma invests each smooth muscle cell. Embedded within this membrane are small granules of silver. × 7,500.

FIG. 3. Section through the wall of a sinusoidal capillary from the area postrema of a rat which had received silver nitrate for 12 months. Surrounding the capillary, which contains a polymorphonuclear leukocyte and an erythrocyte, is a connective tissue space in which collagenous fibrils are visible. An inner membrane delimits this sheath from the capillary endothelium, and an outer one forms a base upon which the glial cells and their processes rest. Both membranes contain copious quantities of granular silver. In addition to their location in these membranes, silver granules are encountered indiscriminately among the collagen fibers of the loose connective tissue sheath. × 20,000.

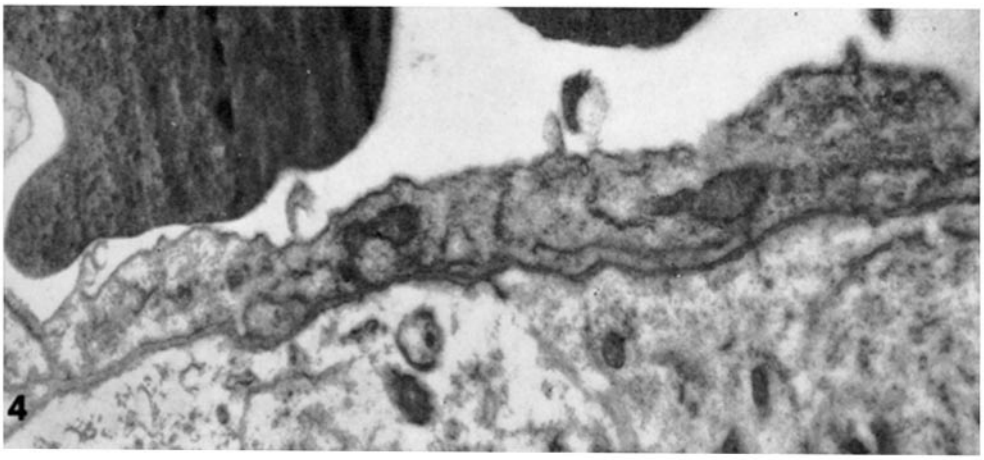


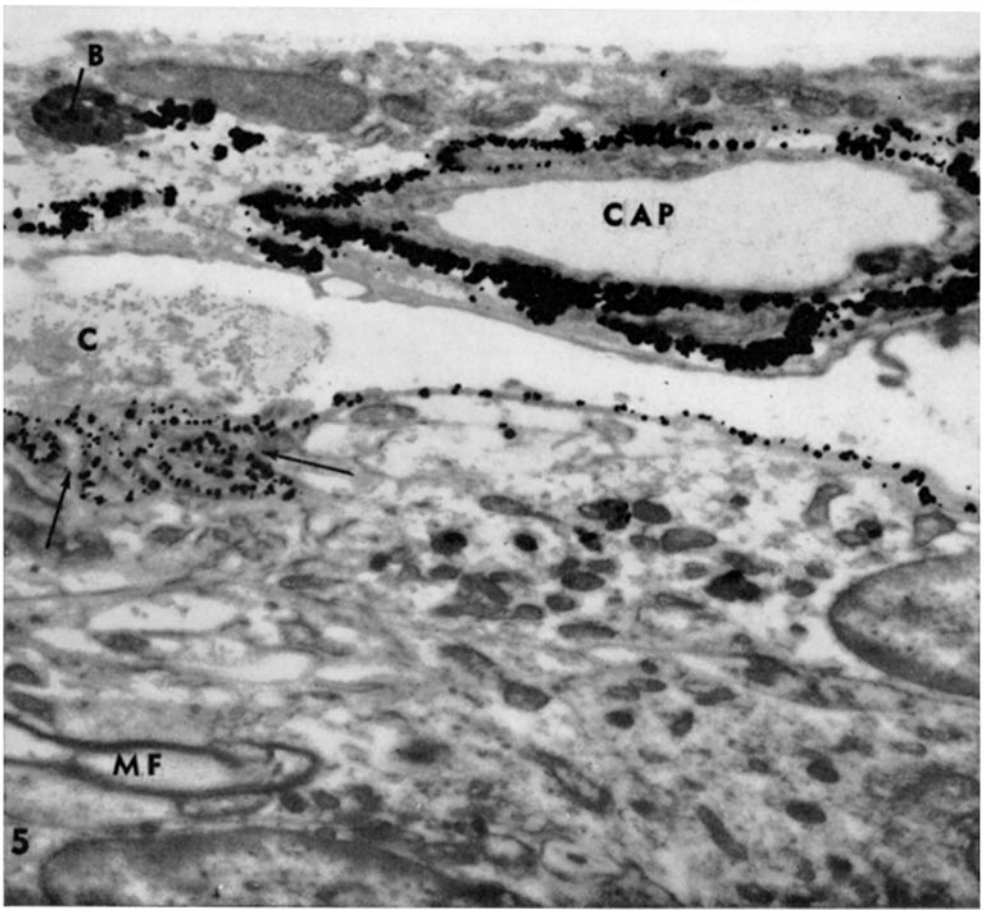
(Dempsey and Wislocki: Electron microscopy of blood-brain barrier)

PLATE 68

FIG. 4. Section through the spinal cord of a mouse which had received silver nitrate in its drinking water for 8 months. Along the upper margin of the figure a capillary vessel and portions of two erythrocytes are visible. Beneath the capillary endothelium, which runs across the center of the figure, is a narrow space bounded by double lines. This space exhibits moderate electron density. It contains no silver deposits, nor is it separated from the parenchyma by a connective tissue sheath. In this location, which is typical of the central nervous system in general, the parenchymal glial cells rest directly upon this delicate endothelial basement membrane. × 30,000.

FIG. 5. Section through the area postrema of a rat which had received silver nitrate for 12 months. The white space at the top of the figure represents the cavity of the fourth ventricle. Forming the wall of the ventricle is an ependymal cell, which is normally quite flattened in this region. A capillary vessel is illustrated (*CAP*) the basement membrane of which contains deposits of silver. Surrounding the vessel is a loose connective tissue sheath in which collagenous fibrils (*C*) are visible. The outer limit of this sheath consists of a membrane, also containing silver deposits, upon which the glial cells rest. Irregular projections of this membrane form interdigitations with glial processes in the region marked with the arrows. A myelinated nerve fiber (*MF*) is visible at the lower left corner. In the ependymal cell, at the top left, is a dark body (*B*) containing blank granules. This body is similar to those illustrated by Dempsey and Wislocki (1955) in other tissues, and is presumably derived from a mitochondrion (see also Fig. 7). × 10,000.



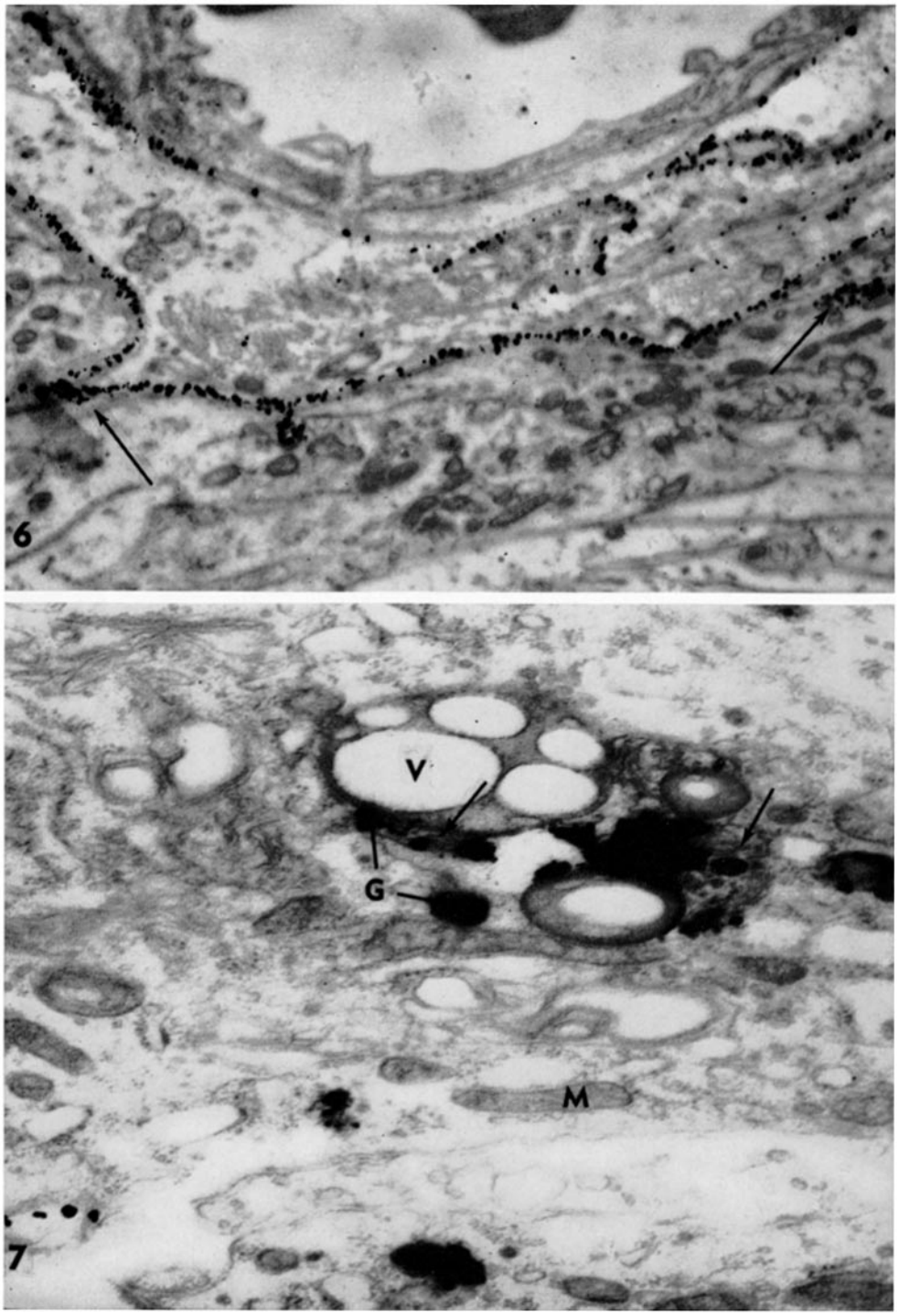


(Dempsey and Wislocki: Electron microscopy of blood-brain barrier)

PLATE 69

FIG. 6. Section through the intercolumnar tubercle of a rat which had received silver nitrate for 12 months. A capillary, with portions of two erythrocytes, is shown at the top of the figure. Surrounding the vessel is a connective tissue sheath with inner and outer limiting membranes, between which are cellular elements and collagenous fibrils. Silver deposits are located in both membranes as well as indiscriminately among the components of the sheath. Here and there projections of the outer membrane interdigitate with glial processes (arrows). × 10,000.

FIG. 7. Section through a glial cell located in the intercolumnar tubercle in a region similar to that illustrated at the bottom of Fig. 6. Occasional dark bodies are encountered in such cells (*cf.* Fig. 5). These bodies frequently exhibit vacuoles (*V*), silver granules (*G*), and, occasionally, membranous structures (arrows) which presumably are derived from the internal folds of mitochondria (*M*). × 50,000.

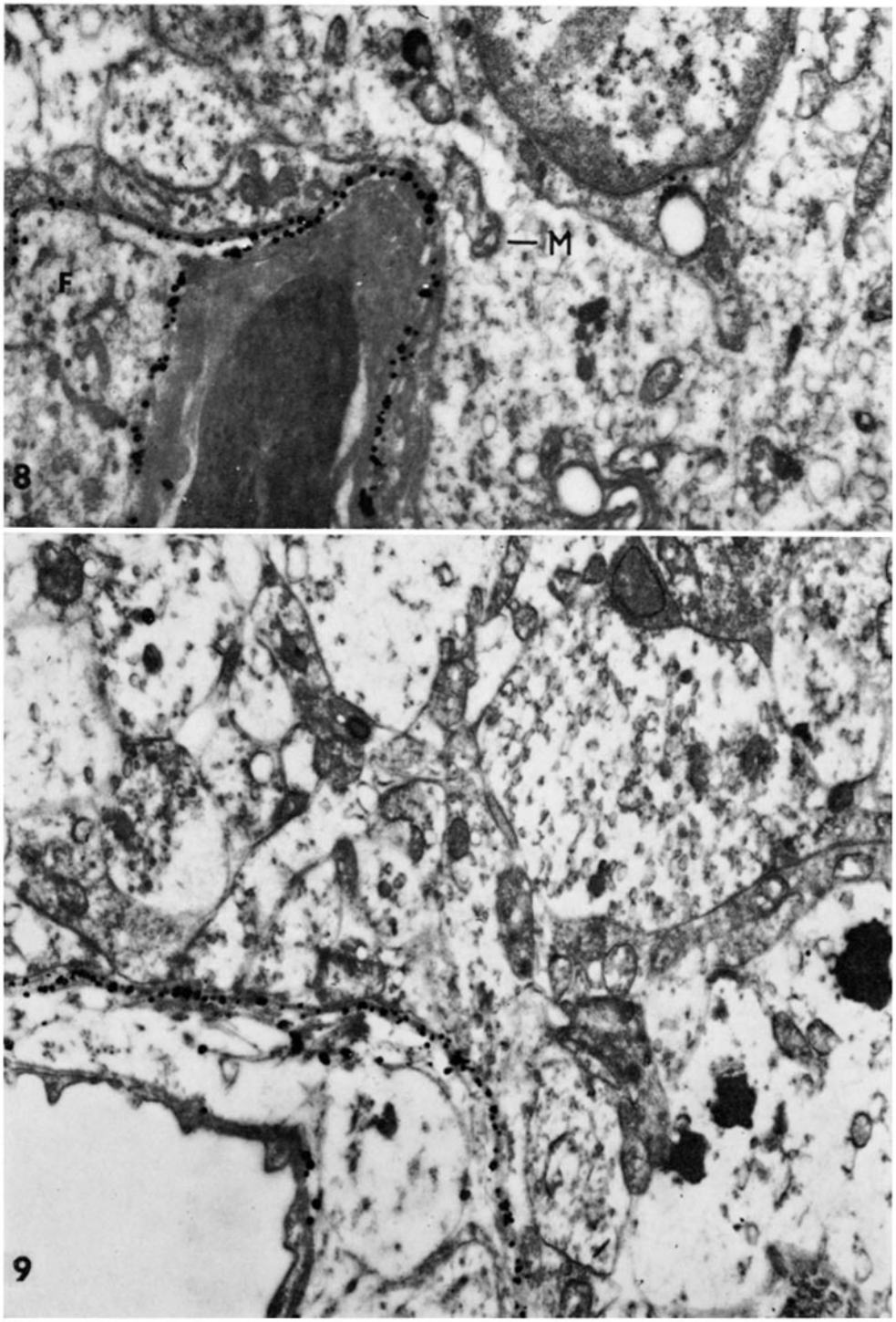


(Dempsey and Wislocki: Electron microscopy of blood-brain barrier)

PLATE 70

FIG. 8. Section through the pineal body of a rat which had received silver nitrate for 12 months. A capillary with a contained erythrocyte is visible at the lower left. Surrounding the vessel is a partially fused double membrane which contains numerous granules of silver. A portion of a fibroblast (*F*) is seen between these membranes. The parenchymal cells exhibit various vacuolar and membranous structures, and their mitochondria (*M*) are considerably larger than those of the fibroblast. × 15,000.

FIG. 9. Section through the neurohypophysis of a rat which had received silver nitrate for 12 months. A sinusoidal capillary, with a characteristic connective tissue sheath, is visible at the lower left of the figure. The membranous margins of the sheath contain silver granules. The irregular, black objects at the lower right are the osmiophilic droplets which characterize the pituicytes. Various vesicular and membranous structures are illustrated in the parenchymal cells. × 10,000.



(Dempsey and Wislocki: Electron microscopy of blood-brain barrier)