SILVER DEPOSITION IN THE CENTRAL NERVOUS SYSTEM AND THE HEMATOENCEPHALIC BARRIER STUDIED WITH THE ELECTRON MICROSCOPE*‡

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The concept that a selective physiological barrier exists between the blood and the brain in the normal animal has become accepted since it has been shown that certain substances circulating in the blood stream do not enter nerve tissue. This phenomenon can be demonstrated by the systemic injection of vitally staining acid aniline dyes, such as trypan blue (Goldmann, 1913). Most of the brain remains unstained, whereas the dura mater, the cerebral vessels, area postrema, the supraoptic crest, the intercolumnar tubercle, the neurohypophysis and stalk, the pineal body, and the choroid plexuses are deeply stained by trypan blue. The same is true for vital staining with silver nitrate, with the difference that silver precipitate occurs in the perivascular stroma only, whereas trypan blue also accumulates within cells (Wislocki and Leduc, 1952). The functional significance of the hematoencephalic barrier is realized in the possible mediation by the barrier structures of nutritional substances, pharmacologic agents, and toxins from the blood into the central nervous system.

There is some controversy concerning the site of the barrier. Some investigators have assigned it to the endothelial cells of the blood vessels (Friedemann and Elkeles, 1934; Spatz, 1934; Broman, 1940; Broman and Lindberg-Broman, 1945) in spite of the fact that perivascular microglia in the cerebrum at times take up particles of the injected dyes. Others hold that the neuroglial cell membranes adjacent to the perivascular space are capable of controlling the passage of substances from the blood vessels (Hauptmann and Gärtner, 1932). It was suggested by Wislocki and Leduc (1952) that the hematoencephalic barrier is composed of a succession of thresholds, different levels of which inhibit the passage of different substances.

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In the investigations reported here it was our purpose to study the hematoencephalic barrier as it pertains to the deposition of silver, using the electron microscope for detailed study of the structures involved.

Material and Methods

In this study we have followed the procedure of Gatz (1949) and Wislocki and Leduc (1952) in the vital administration of 0.5 per cent aqueous solution of silver nitrate to rats as their drinking water over periods of 6 to 8 months. Since the silver deposition is accumulative, more silver precipitate was found in the tissues after 10 to 15 months (Wislocki and Leduc, 1952).

For orientation, serial sections were made of rat brain and stained with Gomori's trichrome stain (hematoxylin, chromotrope 2R, light green, with phosphotungstic acid and acetic acid). For study with the phase contrast microscope and the electron microscope tissues were fixed in 2 per cent osmium tetroxide in Tyrode's solution (pH 7.4). Samples were taken from the cerebrum, cerebellum, medulla, area postrema, and choroid plexus. After fixation, the tissues were washed in Tyrode's solution and briefly in distilled water, dehydrated in ethyl or methyl alcohol, and embedded in methacrylate. Sectioning was done with a cantilever microtome (Porter and Blum, 1953) at 0.025 to 0.05 micron for the electron microscope and at 0.5 to 1.0 micron for the phase microscope. Use of the phase microscope aided in identifying tissues observed with the electron microscope. A Philips electron microscope (EM-100) was used.

OBSERVATIONS

Vital staining with silver lends itself very well to electron microscopic technique. The metal scatters electrons, producing an easily identified image of the metallic precipitate. The silver particles (possibly silver proteinate) vary in size from 10 mμ to 170 mμ in the sections studied.

Location of the Silver.—In rats administered silver nitrate, precipitated silver was found in the perivascular spaces of the choroid plexus (Fig. 1) and the area postrema (Figs. 2 and 3). The silver was deposited on the outer surfaces of the endothelial cells, on the collagen fibrils of the stroma, and on the vessel side of the cell membranes adjacent to the perivascular spaces. A few silver particles were found apparently within endothelial cells in the choroid plexus and area postrema. Very little silver appeared around the capillaries in the cerebrum, cerebellum, and most of the medulla. However, in the part of the medulla bordering the area postrema noticeable quantities of silver occurred perivascularly in the stroma and on cell surfaces (Fig. 4). The silver may have passed through the capillary walls of this part of the medulla, or perhaps it migrated to this location from the area postrema *via* the perivascular spaces. In either case, it is significant that no silver particles were found beyond the neuroglial cell membranes adjacent to the perivascular spaces. This was best demonstrated in the medulla (Fig. 4), though it was true also in the cerebrum and cerebellum in which minute quantities of silver were found.

Nature of the Cell Membranes.—The cell membranes adjacent to the blood vessels are noticeably thicker than the other cell membranes. Their double

wall is apparent on the epithelial cell in Fig. 1 (choroid plexus), on the neuroglial cells in Figs. 2 and 3 (area postrema), and especially on the more highly magnified glial cells of Fig. 4 (medulla). Other glial membranes not adjacent to the perivascular spaces do not have the marked double wall as seen on the surface of the glial "end feet," though they demonstrate the commonly seen double wall between cells. It may also be noted in the more highly magnified pictures (Fig. 4) that some of the silver particles have precipitated between the outer lamella of the neuroglial membrane, and the inner lamella.

Cytology.—In the cells in our illustrations the largest cytoplasmic inclusions are mitochondria (*M*), being quite dense in our preparations, perhaps because of the tonicity of the fixative. In the epithelial cell of the choroid plexus (Fig. 1) there is a concentration of endoplasmic reticulum (*ER*) in the cytoplasm next to the nucleus (*N*). Endoplasmic reticulum is also seen in the neuroglial cells of the area postrema and in the glial cytoplasm in the medulla.

The epithelial cells of the choroid plexus are characteristically covered by bleb-like structures on their surfaces exposed to the ventricle. Fig. 1 shows a ventricular channel which is lined with or filled with these blebs. Their content is homogeneous and of low electron density.

DISCUSSION

The silver nitrate given to rats in this study was dissolved in distilled water, in which it ionized. The chemical fate of the silver ion in respect to its manner of absorption or transport in the blood stream is unknown. As seems to be the case with injected aniline dyes, silver ion may bind chemically with protein in the blood, in a soluble form. It is supposed that the blood-tissue barriers mediate this material as they do other substances in the blood. It is probable that the silver proteinate is relatively unstable, so that silver precipitates from it wherever it "stagnates," as in the perivascular spaces of the area postrema. Our study does not determine whether silver persists in ionic form either in the blood or in surrounding tissues.

The results of our investigations indicate that, in the cerebrum, cerebellum, and medulla, the neuroglial cell membrane surrounding the blood vessels is a definitive site of the blood-brain barrier as it is concerned with deposited silver. It is also apparent that the endothelial lining of the capillaries in the cerebrum, cerebellum, and most of the medulla greatly retards or can completely inhibit the passage of silver particles. In the long periods of time that the rats were exposed to the silver nitrate, some silver may have migrated in the perivascular spaces throughout the brain, especially the short distance from the area postrema into the neighboring parts of the medulla. Nevertheless it is conversely possible that the very small amounts of silver found in the brain did pass through the endothelial cells at the site where they were found in the tissue sections; and it is possible that the endothelial cells of the vessels in the

part of the medulla bordering the area postrema are more permeable to the silver particles than those in the rest of the brain. Where silver does appear in the perivascular spaces in the brain, it is significant that its further migration into the tissue is stopped by the glial cell membranes adjacent to the blood vessels, as shown in Fig. 4.

Two levels of the hematoencephalic barrier are demonstrated in our investigations. The endothelial linings of the vessels in the cerebrum, cerebellum, and medulla constitute the first threshold of the blood-brain barrier. The cell membranes facing the perivascular spaces form the second threshold of the hematoencephalic barrier, as follows:—the perivascular neuroglial cell membranes in the cerebrum, cerebellum, and medulla (blood-brain barrier); the membranes of the neuroglial cells in contact with the blood vessels in the area postrema (blood-brain barrier); the perivascular membranes of the epithelial cells of the choroid plexus (blood–cerebrospinal fluid barrier). Wislocki and Leduc (1952) suggest a third threshold as demonstrated by the administration of trypan blue. The dye accumulates in the epithelial cells of the choroid plexus but does not pass into the cerebrospinal fluid, being retained within the peripheral cell membranes, the third level of the barrier.

In Fig. 1 are illustrated the blebs which were consistently found in the choroid plexus, covering the epithelial cell surfaces which were exposed to the ventricle. In other sections studied, epithelial cells projecting into the ventricle had "foamy" surfaces formed by the blebs. A few of the blebs appeared to have broken away from the cell surface. This may have been the result of a natural phenomenon or a preparative artifact. In groups of these epithelial cells, ventricular channels between the cells are lined with or filled with the blebs, as illustrated in Fig. 1. It may be that these are constant structures with the function of increasing the cell surface area. Alternatively it is possible that these are secretory droplets that are formed as blebs at the cell surfaces and are eventually broken off into the cerebrospinal fluid.

SUMMARY

For the purpose of studying the hematoencephalic barrier as it is concerned with silver circulating in the blood stream, silver nitrate was vitally administered to rats in their drinking water over periods of 6 to 8 months. The cerebrum, cerebellum, medulla, area postrema, and choroid plexus were prepared for light and electron microscopy. Silver deposition was found in the perivascular spaces in the choroid plexus, area postrema, in the medulla surrounding the area postrema, and in minute quantities in the cerebrum, cerebellum, and most of the medulla. Two levels of the hematoencephalic barrier were apparently demonstrated in our investigations. The endothelial linings of the vessels in the cerebrum, cerebellum, and medulla constitute the first threshold of the hematoencephalic barrier (specifically here, blood-brain barrier). The cell mem-

branes adjacent to the perivascular spaces form the second threshold, as follows:—the neuroglial cell membranes in the cerebrum, cerebellum, and medulla (blood-brain barrier); the membranes of the neuroglial cells in the area postrema (blood-brain barrier); and the membranes of the epithelial cells of the choroid plexus (blood–cerebrospinal fluid barrier). This study deals with silver deposition and does not infer that the penetration of ionic silver, if present in the blood stream, would necessarily be limited to the regions described.

Bleb-like structures were observed to cover the epithelial cell surfaces in the choroid plexus. They may be cellular projections increasing the cell surface area or they may be secretory droplets.

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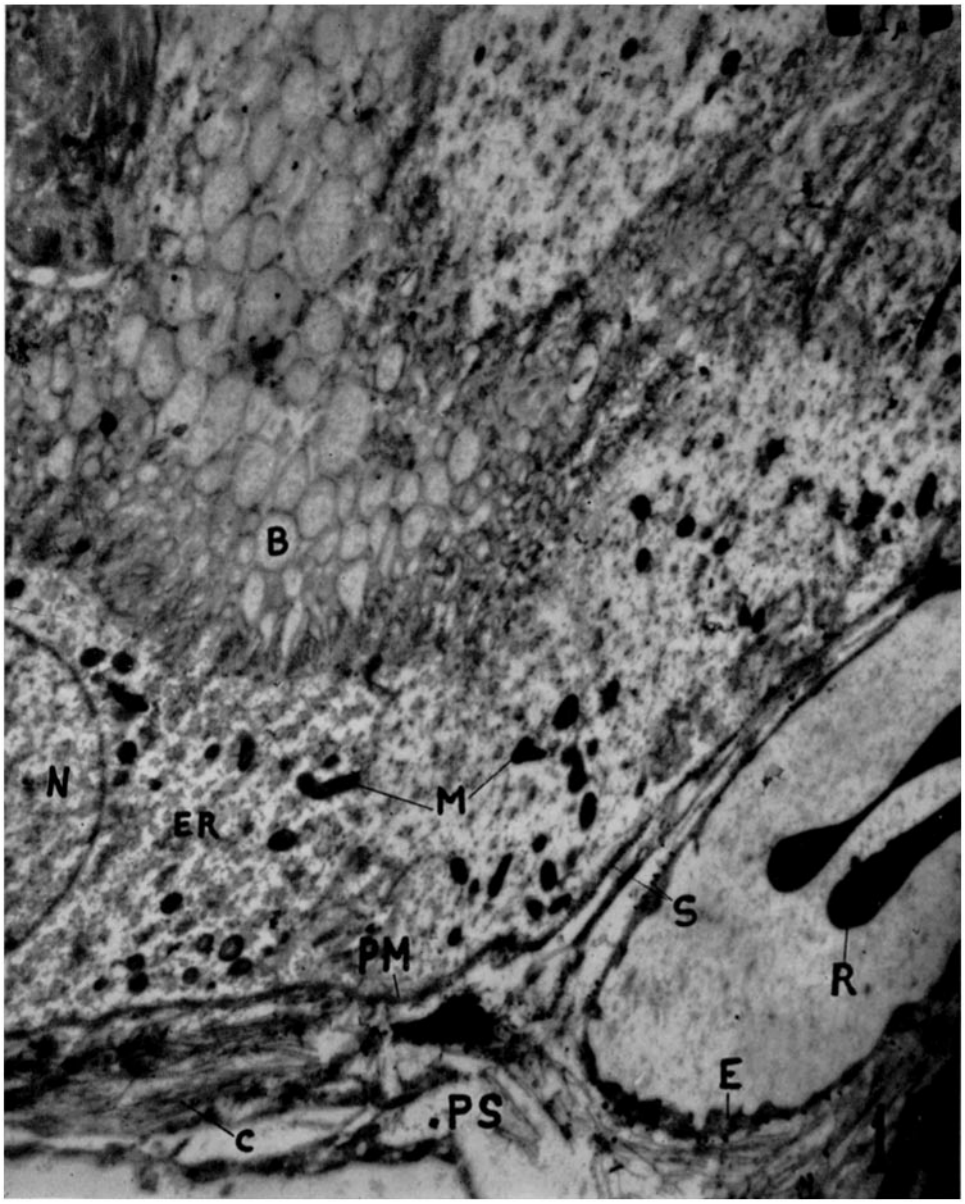
EXPLANATION OF PLATES

PLATE 44

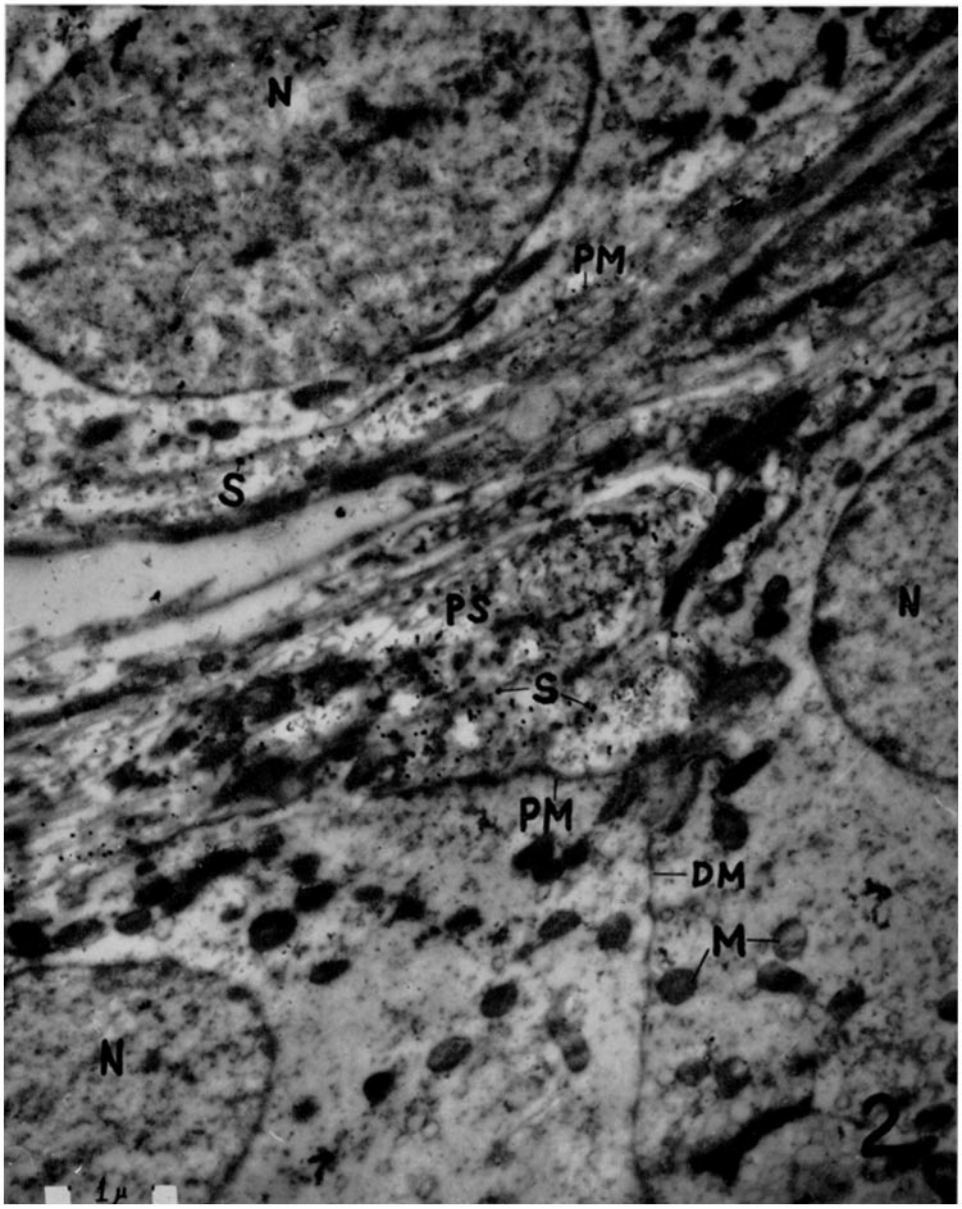
FIG. 1. Choroid plexus. Silver particles (*S*) deposited in the perivascular space (*PS*), on endothelial cell (*E*), on collagen fibrils (*C*), and on the perivascular membrane of the epithelial cell (*PM*). Epithelial cell nucleus (*N*), mitochondria (*M*), endoplasmic reticulum (*ER*), blebs (*B*) at the ventricular surface of the epithelial cell, and erythrocyte (*R*) are indicated. × 7,975.



(van Breemen and Clemente: Hematoencephalic barrier)

PLATE 45

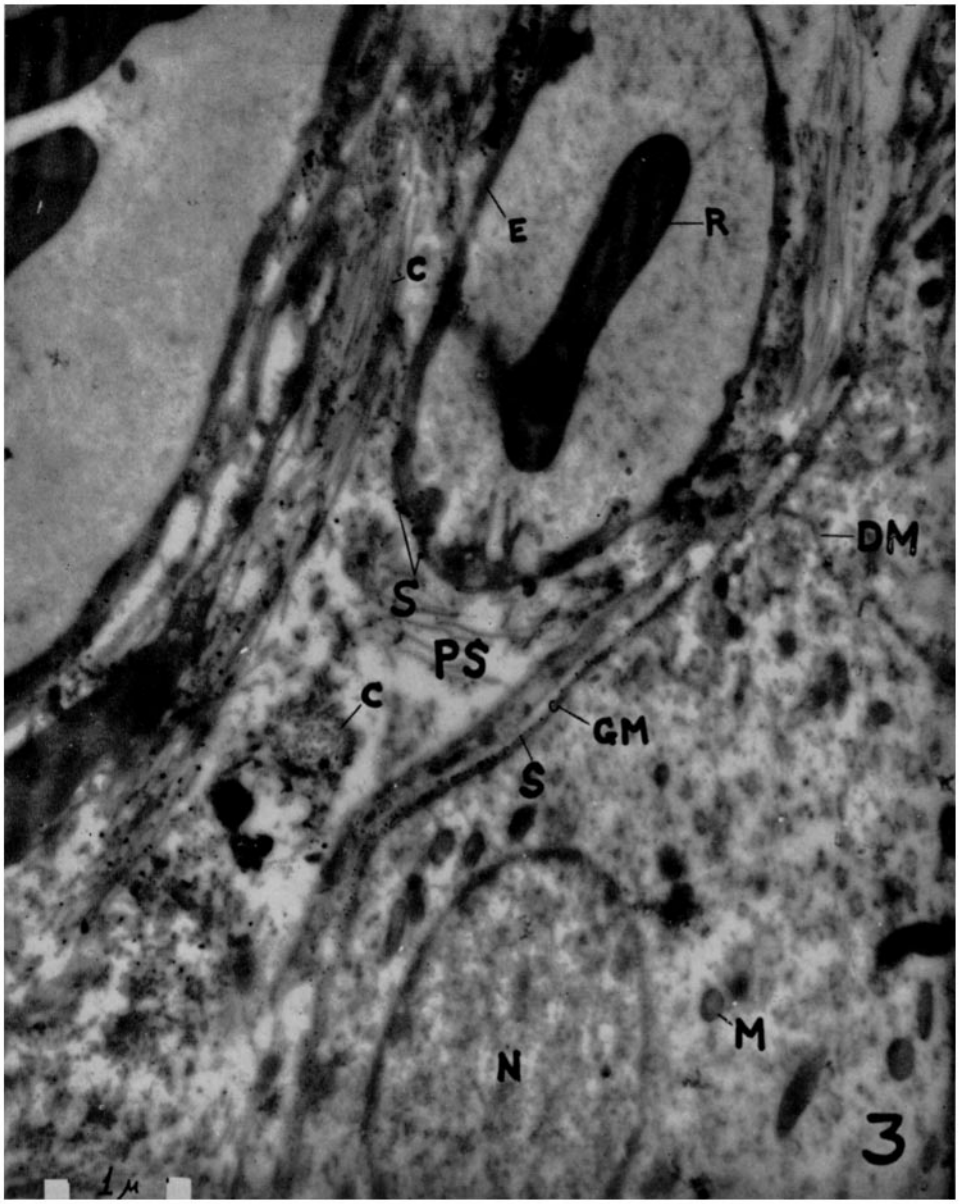
FIG. 2. Area postrema. Silver (*S*) deposited in the perivascular space (*PS*), on collagen fibrils, and on perivascular membranes (*PM*) of the glial cells. Glial nuclei (*N*), mitochondria (*M*), and intercellular double membrane (*DM*) are indicated. × 10,600.



(van Breemen and Clemente: Hematoencephalic barrier)

PLATE 46

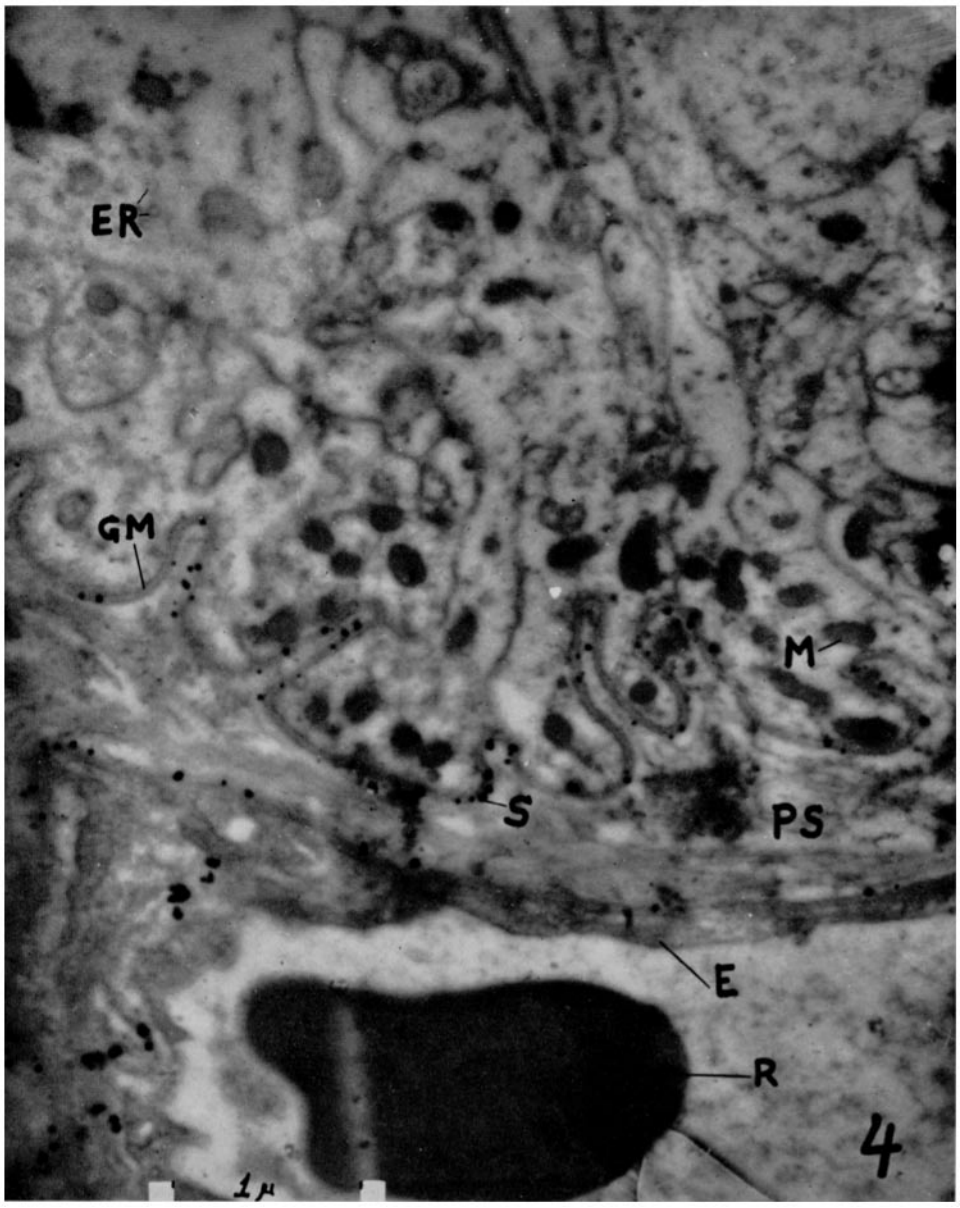
FIG. 3. Area postrema. Silver (*S*) deposited in the perivascular space (*PS*), on endothelial cell (*E*), on collagen fibrils (*C*), and on perivascular membranes (*GM*) of the glial cells. Glial cell nucleus (*N*), mitochondrion (*M*), intercellular membrane (*DM*), and erythrocyte (*R*) are indicated. × 12,650.



(van Breemen and Clemente: Hematoencephalic barrier)

PLATE 47

FIG. 4. Medulla. Glial "end feet" on blood vessel. Silver (*S*) deposited in the perivascular space (*PS*), on endothelial cell (*E*), on collagen fibrils, and on perivascular glial cell membranes (*GM*). Erythrocyte (*R*), mitochondrion (*M*), and endoplasmic reticulum (*ER*) are indicated. × 24,500.



(van Breemen and Clemente: Hematoencephalic barrier)