Hemosiderin Granules in the Choroid Plexus.*

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Since the first light microscope descriptions of the choroid plexus by Faivre (1) and Luschka (2), numerous kinds of granules and vacuoles have been described as components of epithelial cells of the choroid plexus, such as slightly pigmented, osmiophilic granules (3), hyaline-like granules (4), and fuchsinophilic and basophilic granules (5). These observations were promptly interpreted as evidence of secretory activity. Evidence in partial support of this interpretation was not lacking. Cappelletti (6) had found that injection of pilocarpine increased the flow of spinal fluid while Pettit and Girard (4), later confirmed by Meek (7), showed that after the injection of ether, theobromine, muscarine, or pilocarpine, the apical ends of the choroidal epithelial cells became more watery and increased in height. Later, Dandy and Blackfan (8) produced a unilateral hydrocephalus by plugging the foramen of Monro on one side, and Flexner (9) showed that energy is required for the production of spinal fluid.

Today, production of the cerebrospinal fluid by the choroid plexus is generally accepted as the most plausible theory. A few investigators, however, have strongly opposed this theory, believing instead, that the spinal fluid is produced by the parenchyma of the brain and percolates via perineuronal and pericapillary spaces into the ventricles and subarachnoid space. Foremost in holding this theory were Hassin (10–13) and Hassin, Oldberg, and Tinsley (14). These men considered the choroid plexus to be an organ of absorption, and believed that it removed noxious and waste substances from the blood. They insisted that what other observers had interpreted as secretion granules and vacuoles in the cytoplasm of the choroid epithelial cells were actually catabolic products which had been picked up by these cells for disposal.

In the course of a comparative study of the ultrastructure of the choroid plexus in the rat, rabbit, guinea pig, opossum, dog, and hamster, "frothy" bodies of varying size were observed constantly in the guinea pig but were not found in any of the other animals.

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Materials and Methods

The animals reported on here are two groups of adult guinea pigs in apparent good health. The first group consisted of three animals and the second group, sacrificed 18 months after the first, contained two animals. All animals were sacrificed in the same manner by being placed under deep anesthesia with nembutol and, after reflecting the scalp and temporalis muscles laterally, were beheaded to allow rapid exsanguination of the brain, and the lateral and fourth ventricles were opened to expose the plexuses. Immediately as the plexuses came into view, 1 per cent buffered osmic acid (15), chilled to about 4°C. was dripped onto the tissue *in situ* for 10 minutes. Following this period of prefixation the tissue was removed and placed in a bath of fresh 1 per cent osmic acid and allowed to come to room temperature during a further fixation time of 30 minutes. The average time from decapitation to the first drop of fixative was about 3 minutes. The tissues were dehydrated in ethyl alcohol and embedded in *n*-butyl methacrylate then sectioned on a Porter-Blum microtome. Sections appearing golden or creamy-silver were selected for examination in the electron microscope. Bits of the plexuses from the last two animals were obtained and fixed with neutral formalin and embedded in paraffin, after which sections were cut at 3 μ and stained by hematoxylin and triosin, lipofuchsin, and Prussian blue techniques for examination by light microscopy.

OBSERVATIONS AND DISCUSSION

Sections from the choroid plexuses of both the fourth and lateral ventricles of each animal were examined in the electron microscope at magnifications ranging from 1,000 to 7,000. In its morphology, the ultrastructure of the ependyma was similar to that described for the rat by Dempsey and Wislocki (16) and Maxwell and Pease (17); for the rabbit by van Breemen and Clemente (18) and Millen and Rogers (19); for the dog by Shryock and Case (20), and for the monkey, opossum and woodchuck by Wislocki and Ladman (21). However, none of the whorled bodies seen by Millen and Rogers (19) were seen in the guinea pig. In each animal, however, large numbers of inclusion bodies ranging in size from 0.5 to 5 μ in diameter were found dispersed in the cytoplasm of the choroidal cells. The larger ones appeared "frothy," with many and variously sized clear vacuolar spaces in them. Most of the bodies were irregularly round or elliptical in shape and in appearance seemed almost

identical to similar bodies seen in phagocytic cells found in the subarachnoid space by Schultz (22). Wislocki and Ladman (21) observed several similar bodies in the choroid plexus of rats fed silver nitrate and believed they represented accumulations of lipofuchsin.

Evidence to indicate that the material forming these bodies had been taken into the cell through the polypoid border was lacking. Likewise, although many of the smaller non-vacuolated bodies were of a size similar to mitochondria, nothing was seen which would indicate that either these or the larger ones had originated from degenerating mitochondria. The bodies varied from 2 to 3 up to 20 to 25 in number seen per cell section. The small dense granules as well as the larger frothy type could be found scattered randomly in either the apical or basal portion of the cell, although the larger bodies seemed to be somewhat more numerous basal to the nucleus.

At first these frothy inclusions, as well as the homogenously dense bodies, were thought to represent lipid-like materials because in thin sections of osmium-fixed material from the methacrylate-embedded tissue examined in the light microscope dark osmiophilic granules were readily observable (Fig. 1). When, however, formalin-fixed, paraffin-embedded material was also examined in the light microscope, brownish colored granules which had not been dissolved out by the fat solvents during embedding were also revealed. Comparison of the granules in the formalin-fixed and in the osmium-fixed material showed the granules to be only slightly osmiophilic, most of the coloration being due to the color of the pigment. Subsequent examination of histochemically stained sections showed the lipofuchsin-stained tissue to be negative while the Prussian blue preparation was positive, thus identifying these bodies as hemosideran granules and confirming work done many years ago by Flather (23), who described large quantities of hemosideran in the choroid plexus of the guinea pig in both normal animals and in increased amounts after the injection of diphtheria antitoxin, atropine, or muscarine. The iron content of the hemosiderin here would account also for the high electron opacity of these granules.

The presence of a blood breakdown product such as hemosideran in an organ such as the choroid plexus, which, though highly vascular, is not considered to be concerned with blood destruction or as a part of the reticulo-endothelial system, has yet to be explained. Since hemosideran is considered to be formed within the cytoplasm of a cell, it would not seem to indicate phagocytosis on the part of these cells. At very least, the presence of such a waste product emphasizes that an attitude of caution is appropriate in interpreting the still controversial and incompletely understood role of the choroid plexus.

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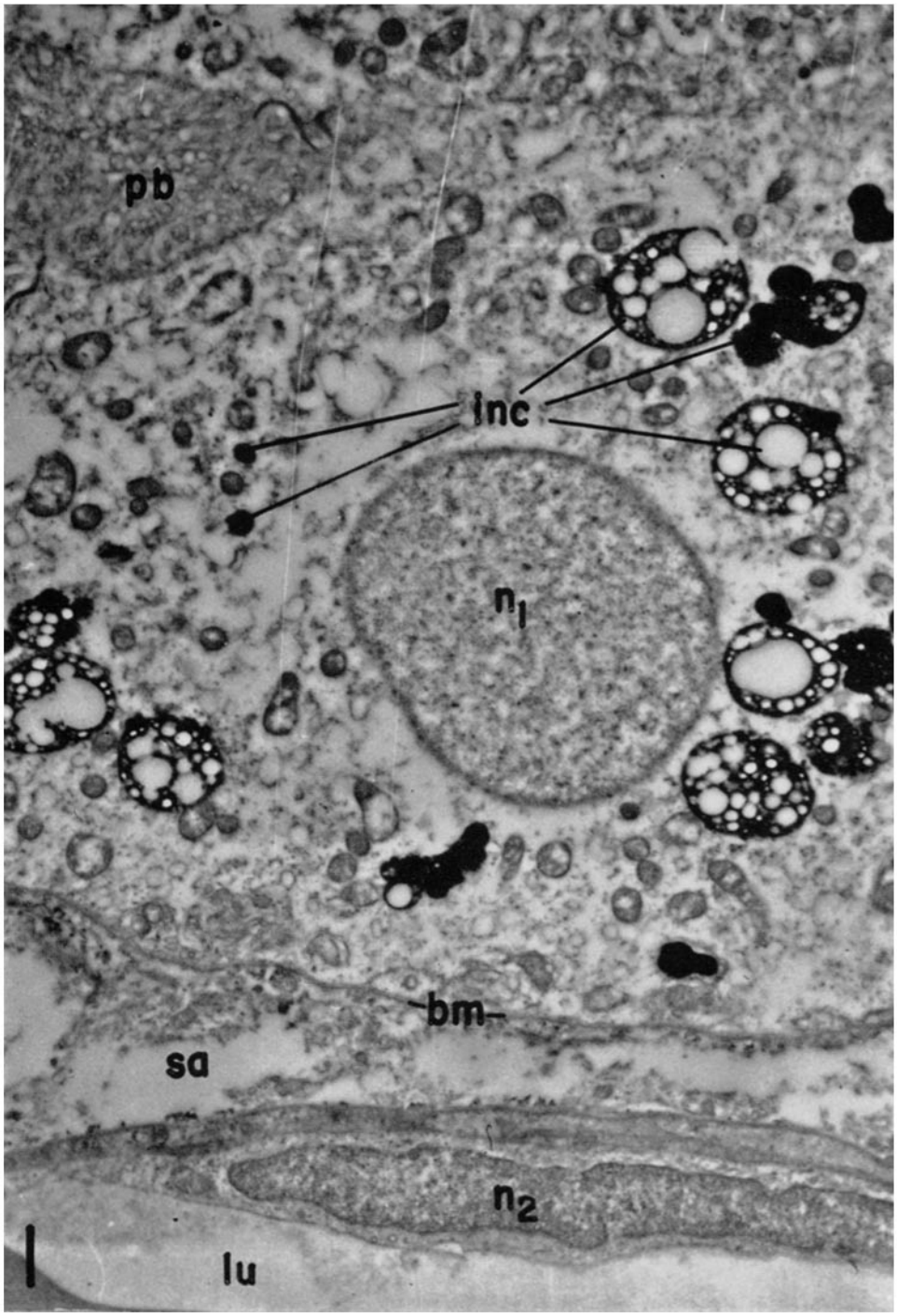

(Case: Choroid plexus)

EXPLANATION OF PLATE 254

PLATE 254

FIG. 1. Guinea pig: choroid plexus of the 4th ventricle. Surrounding the nucleus (n_1) of this ependymal cell are many inclusion bodies (*inc*) of hemosideran representing extremes in size, from small dense granules to large frothy bodies. The latter were found only in the guinea pig. Also indicated are the polypoid border (*pb*), the basement membrane (*bm*), the subarachnoid space (*sa*), and the nucleus of an endothelial cell (n_2) bulging into the lumen (*lu*) of a capillary. × 6,200.