FINE STRUCTURE OF THE NEUROHYPOPHYSIS OF THE OPOSSUM (*DIDELPHIS VIRGINIANA*)

LAWRENCE M. ROTH, M.D., and SARAH A. LUSE, M.D.

From the Departments of Anatomy and Pathology, Washington University School of Medicine, St. Louis

ABSTRACT

The neurohypophysis of the opossum (*Didelphis virginiana*) was studied by electron microscopy in order to amplify Bodian's classic light microscopic observations in which he demonstrated a definite lobular pattern. The lobule of the opossum neurohypophysis is divided into three regions: a hilar, a palisade, and a septal zone. The hilar portion contains bundles of nerve fibers, the extensions of the hypothalamo-hypophyseal tract containing neurofilaments but few neurosecretory granules. In the opossum, pituicytes have a densely fibrillar cytoplasm. Herring bodies are prominent in the hilar region. They are large bodies packed with neurosecretory granules that have been described as end bulb formations of axons. From the hilar region, axons fan out into a palisade zone where the nerve terminals packed with neurosecretory granules, mitochondria, and microvesicles abut upon basement membranes. The neurosecretory granules are similar to those present in the neurohypophysis of other mammals, except for an occasional huge granule of distinctive type. Material morphologically and histochemically resembling glycogen occurs as scattered particles and as aggregates within nerve fibers. The septal zone, containing collagen, fibroblasts, and numerous small capillaries, is separated from the adjacent glandular tissue by a basement membrane.

The neurohypophysis of the North American opossum (*Didelphis virginiana*) has been studied in detail by Dawson (1), Bodian (2, 3), Wheeler (4), Green (5), and Bargmann (6), and that of the Brazilian large-eared opossum (*Didelphis aurita*) by Hanström (7–9). The opossum is unusual in that no infundibular stem is present and the neurohypophysis rests as a pear-shaped body within the basket-shaped pars distalis, separated from it only by the layer of the pars intermedia, one cell thick, and by the lumen of the hypophyseal cleft (9). Using a variety of histological staining technics, including silver protargol, Gomori's chrome-alum-hematoxylin, and the Mallory-Azan method, Bodian (3) demonstrated that the opossum neurohypophysis is divided into well organized lobules by connective tissue septa. Within some septa, parenchymal invaginations form small islands termed accessory lobules. Lobules are particularly well developed at the periphery of the neurohypophysis, but centrally are distorted by the large bundles of axons from the hypothalamo-hypophyseal tract. Bodian subdivided the lobule into three zones: a hilar region containing nerve fibers from the hypothalamo-hypophyseal tract, a palisade zone of nerve terminals, and a septal zone containing connective tissue and capillaries. In chrome alum-hematoxylin-phloxine–stained sections, there is a concentration of finely granular neurosecretory material at the periphery of the

lobule in the region of the nerve terminals (3, 9). This is not unique to the opossum, as Diepen (10) noted a similar pattern in the mole, the pig, certain primates, and in *Setifer setosus*, a primitive insectivore.

Bargmann and Scharrer's (11) concept that neurosecretory material formed within nerve cells of the supraoptic and paraventricular nuclei of mammals passes through their axons into the posterior lobe of the hypophysis now is widely accepted. Evidence has accumulated that neurosecretory granules, like those of other protein-secreting cells, are formed by the Golgi apparatus (12–14). The question of synthesis of neurosecretory material within the axons themselves, however, remains controversial.

The neurohypophysis of a wide variety of vertebrates already has been studied by electron microscopy as is indicated in the recent review by Diepen (15). In addition, the status of neurosecretion in invertebrates recently has been reviewed by Bern and Hagadorn (16). Because of its relatively simple organization, the opossum's neurohypophysis is ideal for correlation of observations by electron microscopy with those of light microscopy.

MATERIALS AND METHODS

Six adult opossums (*Didelphis virginiana*) were used in this study. Animals were anesthetized with intramuscular pentobarbital sodium. The skull was opened rapidly, the pituitary gland removed, and the neurohypophysis dissected. For light microscopy, pituitary glands were fixed in Zenker-formalin, 10 per cent neutral buffered formalin, and Carnoy's fixative (60 per cent absolute alcohol, 30 per cent chloroform, and 10 per cent glacial acetic acid), and stained with hematoxylin and eosin, aldehyde-thionin, Verhoeff-Van Gieson, Heidenhain's modification of Mallory-Azan, Mallory's aniline blue, and thionin stains, the periodic acid–Schiff-dimedone reaction, and the periodic acid–Schiff reaction after diastase.

For electron microscopy, the neurohypophysis rapidly was cut into approximately 1 mm cubes and placed in Dalton's chrome-osmium fixative for 1 hour at 0°C, dehydrated through graded ethanol solutions, and embedded in Epon-812 according to the method of Luft (17). Thin sections were cut on glass knives in a Porter-Blum microtome, mounted on grids coated with collodion, and stained with lead or uranyl acetate. Grids were examined in a RCA model EMU-3F and photographed at initial magnifications of 1,000 to 17,000 diameters.

OBSERVATIONS

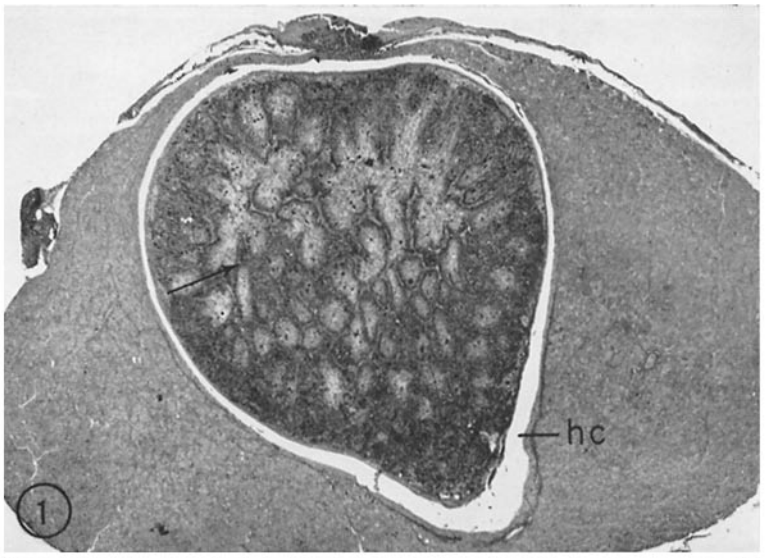
Light Microscopy

The neural lobe of the opossum pituitary is surrounded by a layer of pars intermedia, only one cell thick, and separated from the anterior lobe by the hypophyseal cleft (Fig. 1). The neurohypophysis has a distinct lobular pattern outlined byconn ective tissue septa containing capillaries (Figs. 2 and 3). Adjacent lobules are connected at the hilum by extension of fibers of the hypothalamohypophyseal tract. Large homogeneous accumulations of aldehyde-thionin-positive material, Herring bodies, occur in the hilar regions of the lobules. Some of them, however, have a granular structure with a central zone that does not stain with aldehyde-thionin. Small granules of material stained by aldehyde-thionin are located throughout the lobule and are especially numerous in the palisade zone where they are arranged in a linear pattern perpendicular to the lobular borders as they outline the course of the nerve terminals. Cell bodies of pituicytes are confined to the hilar region. They have indistinct cytoplasmic outlines and round to oval nuclei with occasional nuclear inclusions. The axonal terminals in the palisade

FIGURE 1. Low power micrograph of pituitary of opossum. The neurohypophysis (arrow) is separated from the adenohypophysis by the hypophyseal cleft (*HC*). Aldehyde-thionin stain. × 30.

FIGURE 2 Aldehyde-thionin–stained paraffin section of neurohypophysis of opossum showing the well developed lobules separated by connective tissue septa. Herring bodies are prominent (arrows). The central pale area of each lobule is the hilar zone. The dense peripheral zone is the palisade zone. × 140.

FIGURE 3 PAS -dimedone–stained paraffin section to demonstrate glycogen (arrow). The nuclei are those of pituicytes. × 480.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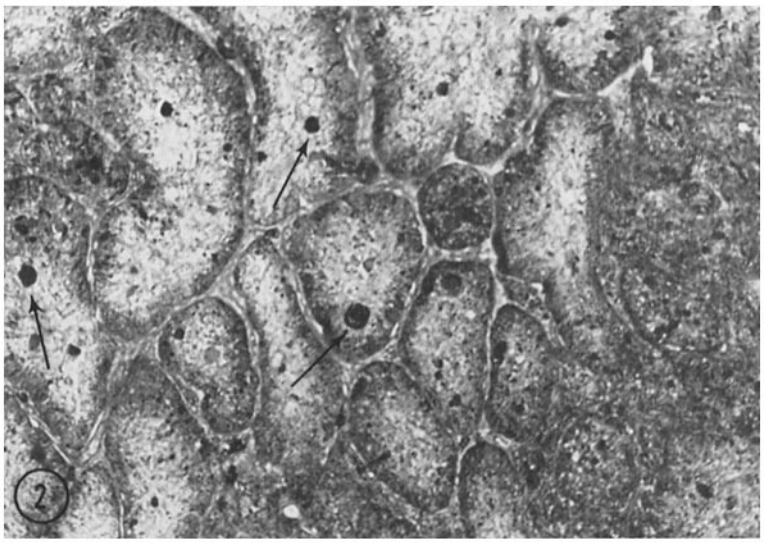
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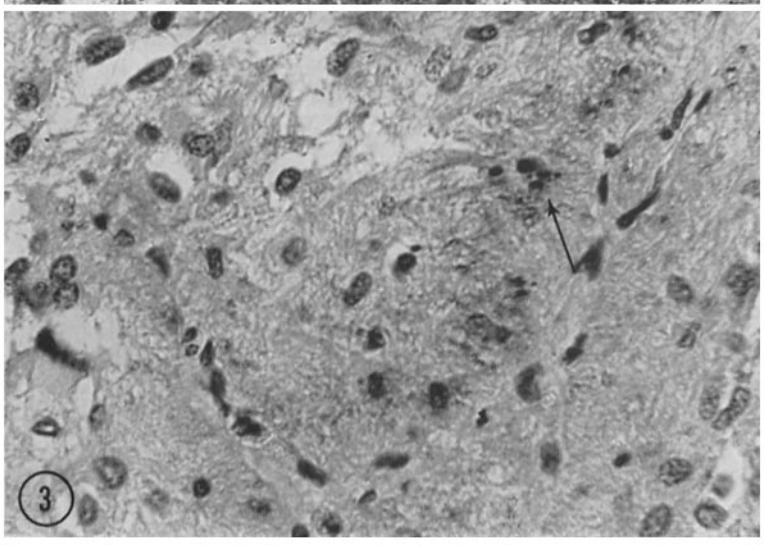
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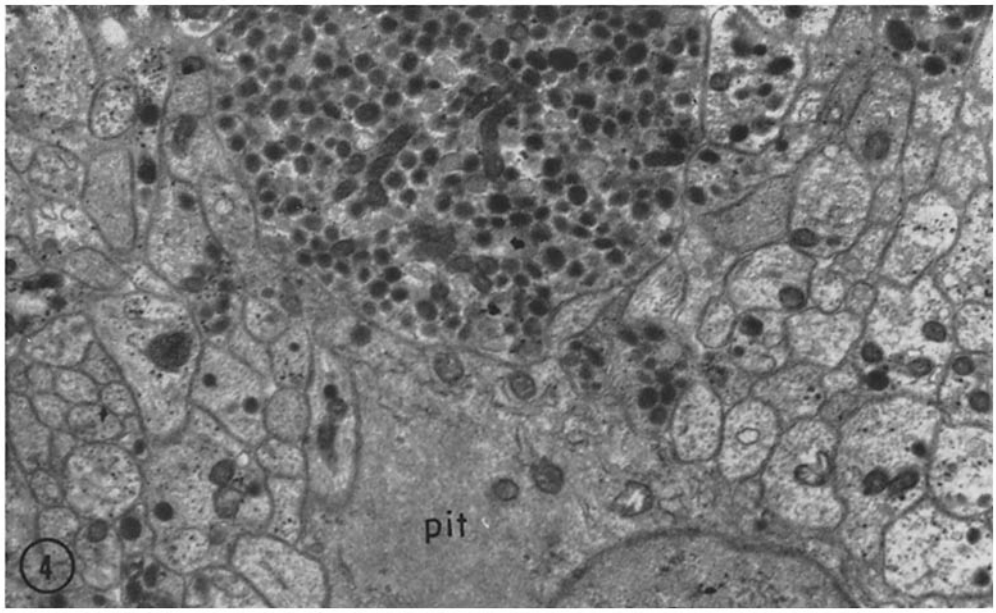
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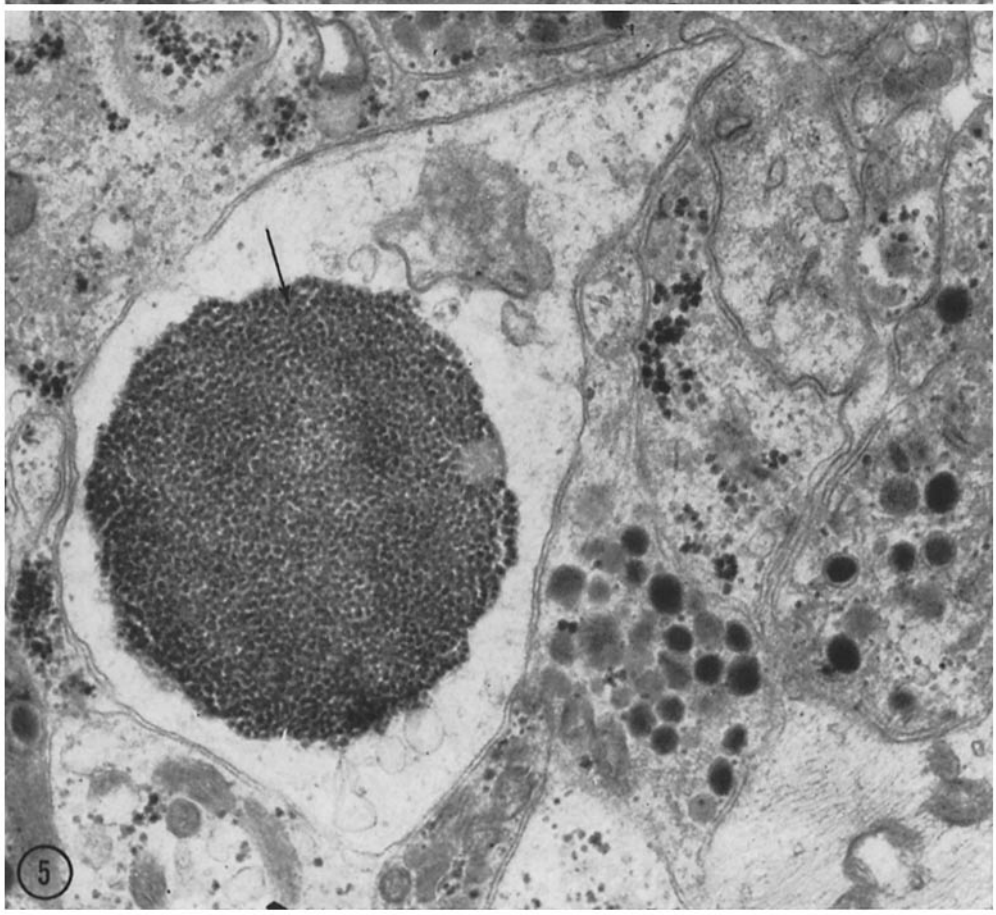
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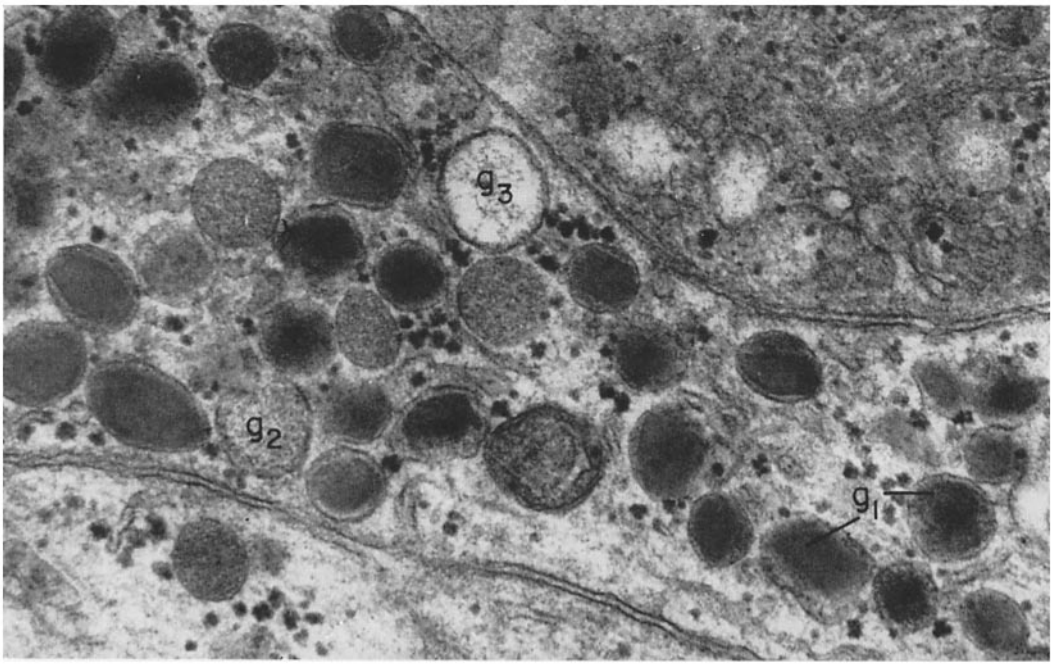


FIGURE 6 This is an axon filled with neurosecretory granules. Some are clearly enclosed by a limiting membrane (g_1). Others are of lesser density and without a distinct membrane (g_2). Some are essentially empty (g_3). Scattered smaller particles are glycogen. Lead stained. × 50,000.

zone are ensheathed by processes extending from pituicytes in the hilar region. The septal zone consists of a connective tissue space composed principally of collagen and capillaries. The accessory parenchymal lobules stand out when its fibrous connective tissue is stained by the Mallory-Azan method.

Electron Microscopy

UNMYELINATED AXONS cut in cross-, oblique, and longitudinal sections form much of the neurohypophysis (Fig. 4). In the hilar region, whole bundles of axons are cut in the same plane and are thought to represent fibers of the hypothalamo-hypophyseal tract. These axons resemble those seen elsewhere in the nervous system in that they contain neurofilaments and mitochondria, but differ from other axons in that many contain granules resembling those found in secretory cells. In addition, electron-opaque particulate material, considered to be glycogen, occurs in small or even large aggregates within the axoplasm (Fig. 5).

NEUROSECRETORY MATERIAL: In the opossum neurohypophysis, similar to that in other mammals, this material consists of small, round to oval granules (Fig. 6). These granules have a

FIGURE 4 Electron micrograph of the hilar zone of the neurohypophysis of the opossum. Numerous unmyelinated axons are evident at both the right and left. Centrally, part of a pituicyte (*pit*) is present and above it a distended axon filled with neurosecretory granules. Lead stained. × 6,000.

FIGURE 5 A large aggregate of glycogen is evident within an axon (arrow). Small scattered particles of glycogen are present in neighboring axons. The section is lead stained. × 25,000.

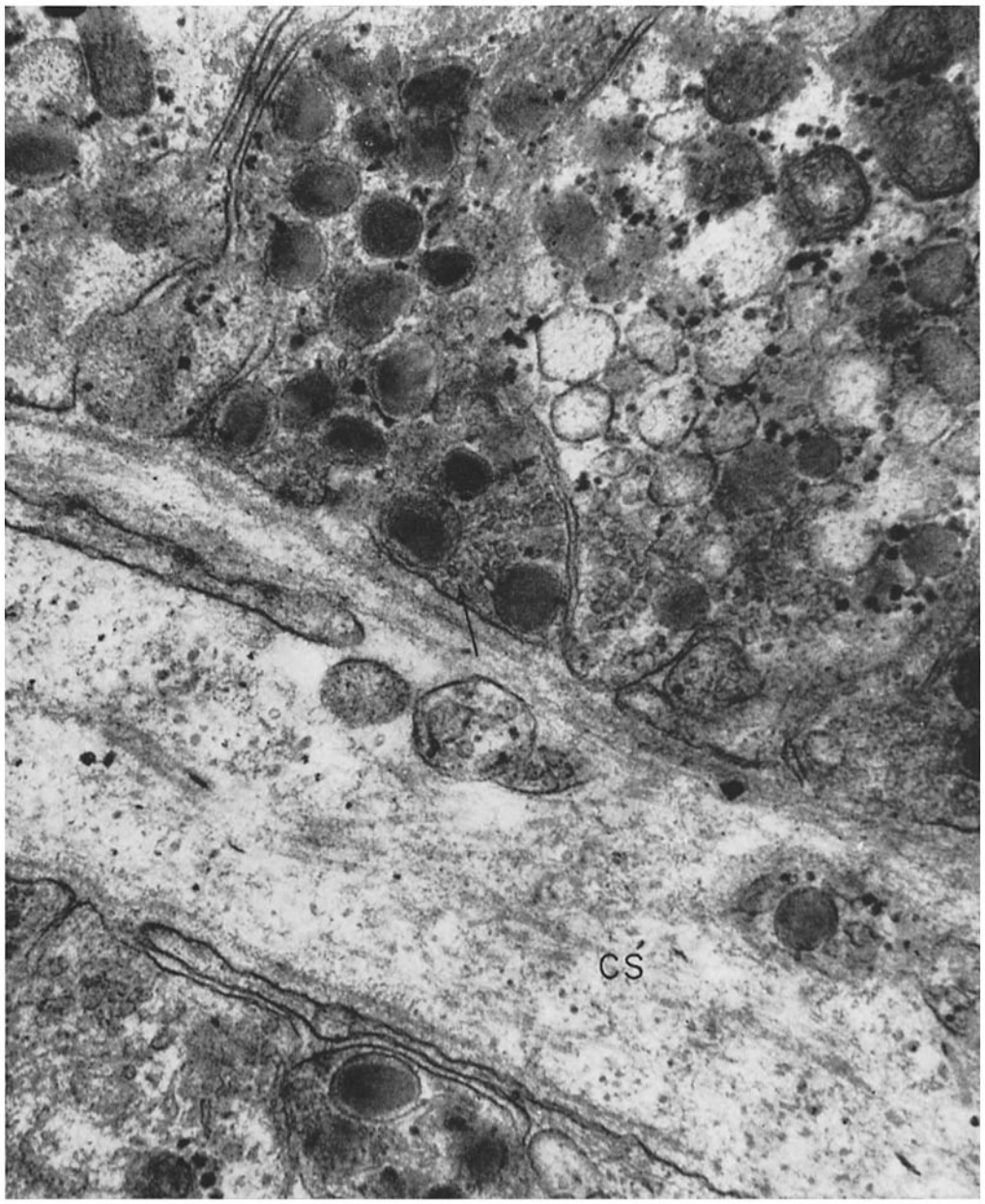
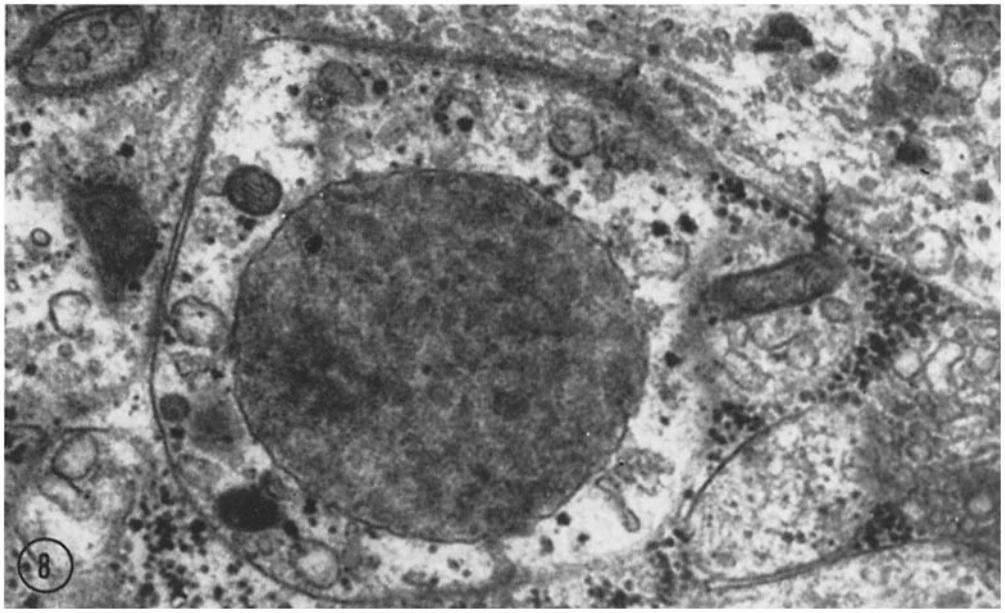


FIGURE 7 This is a section from the palisade zones of two adjacent lobules separated by a connective tissue septum (*CS*). Filled and empty neurosecretory granules are closely packed in the nerve terminals. At the arrow small vesicles resembling those of synapses are evident. Lead stained. × 40,000.

FIGURE 8 This electron micrograph of the hilar zone demonstrates an axon containing a huge granule with a distinct limiting membrane. Glycogen particles are present marginally. Lead stained. × 25,000.

FIGURE 9 This micrograph of the hilar region demonstrates two adjacent Herring bodies that occupy a major portion of the field. At the upper right and lower left are fascicles of unmyelinated axons. Lead stained. × 13,500.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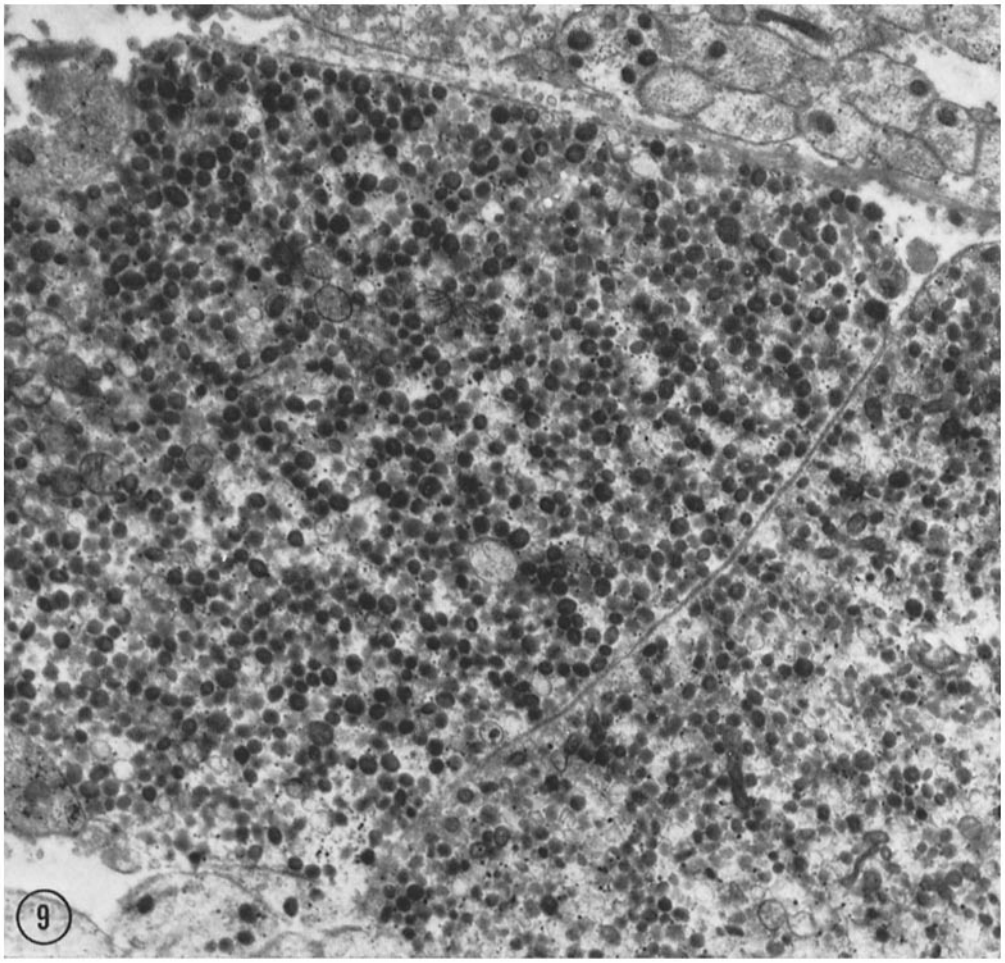
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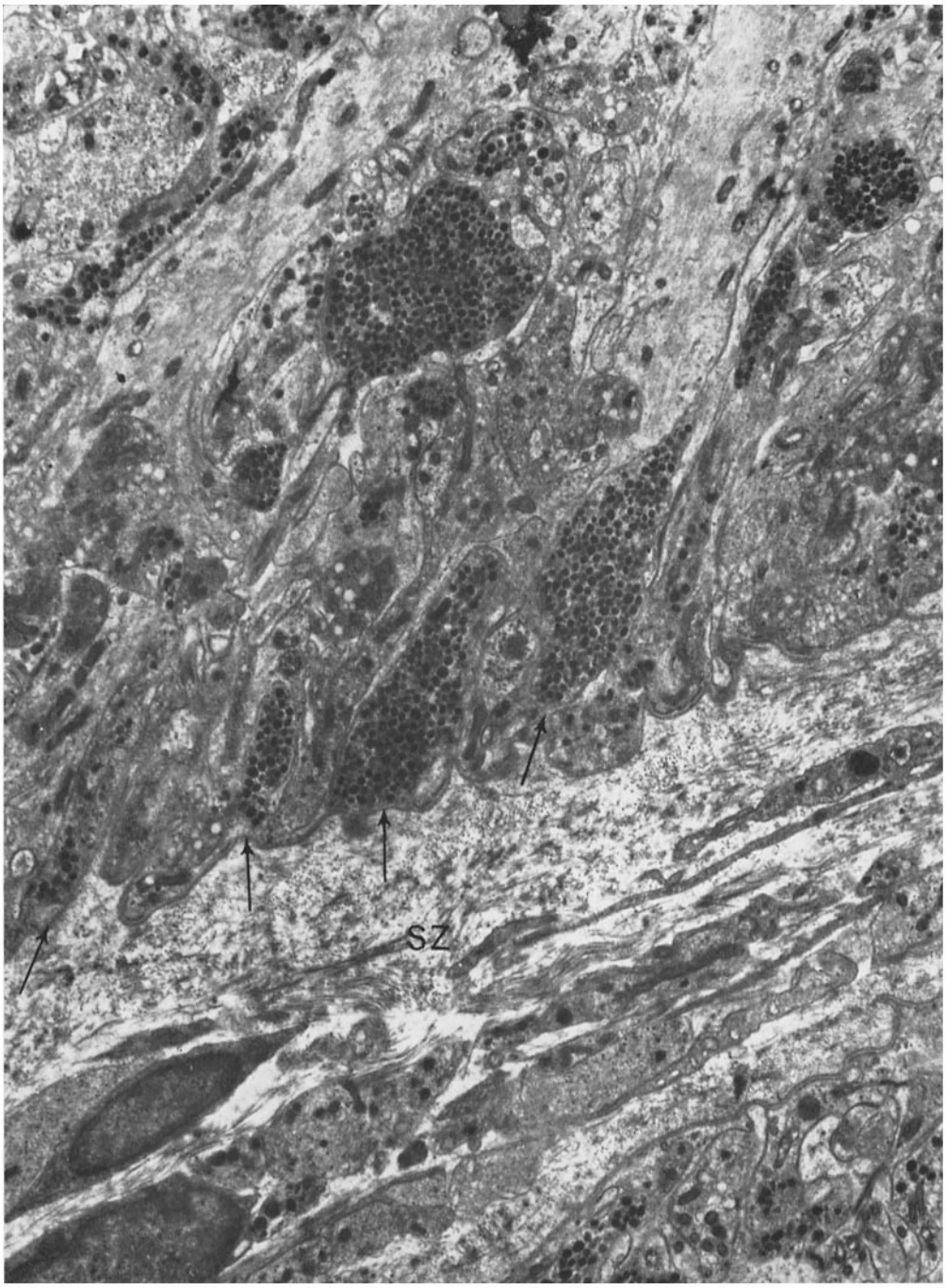

FIGURE 10 Several nerve terminals (arrows) filled with neurosecretory granules are present in linear array forming the palisade zone. At their base they are separated from the septal zone (*SZ*) by a basement membrane. The septal zone contains fibroblasts and collagen. Uranyl stained. × 3,500.

dense central zone separated from an outer limiting membrane by a thin electron-transparent region. Occasional axons and nerve terminals contain a few somewhat electron-transparent granules of the same size. Intermediate forms of moderate density also occur in nerve terminals (Fig. 7). In some regions, granules of similar appearance have no evident limiting membrane and may represent a stage of disintegration. As has been observed by light microscopy, neurosecretory granules are most closely packed in nerve terminals and in pre-

of similar appearance that may be either small Herring bodies or perhaps bulbous axons.

Nerve terminals, the bulbous terminal enlargements of axons, sweep across the palisade zone at right angles to the boundary of the septal zone in favorably oriented sections (Fig. 10). They are packed with neurosecretory granules and occasional mitochondria, and are ensheathed by processes of the pituicytes. Scattered nerve terminals may be less densely packed with either empty granules or a mixture of filled and empty granules.

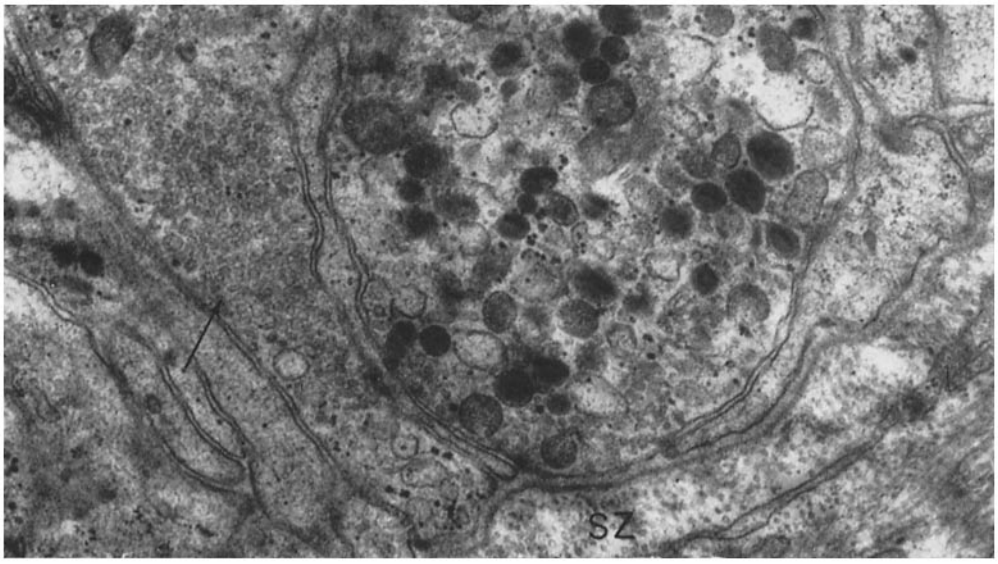

FIGURE 11 The septal zone (*SZ*) in this micrograph is a narrow cleft filled with collagen cut in cross-section. Two nerve terminals are present. The one on the right is distended with granules. The one on the left is filled with vesicles (arrow) of synaptic type. Lead stained. × 20,000.

terminal axons, but also occur in individual axons in the hilar region.

A much larger granule of intermediate electron opacity with a distinct limiting membrane occasionally is present in axons (Fig. 8) and is similar to granules observed by Palay (12) in the preoptic nucleus of the goldfish.

HERRING BODIES: These are large, irregularly shaped structures in the hilar zone (Fig. 9). They are bounded by a plasma membrane and correspond in size and location to bodies stained with aldehyde-thionin. They are packed with neurosecretory granules with only rare interspersed mitochondria. In addition, there are smaller bodies

The limiting membrane of the axonal terminal is focally thickened where it ends on the basement membrane bounding the extracellular space of the septal zone. These terminals contain, in addition to neurosecretory granules, small vesicles morphologically identical to those occurring in synapses elsewhere in the nervous system (Figs. 7 and 11). In lead-stained sections of nerve terminals there are particles identified as glycogen.

NEUROSECRETORY CELLS: In the hilar zone, rare cells occur that have an irregular, eccentric nucleus and a well developed rough-surfaced endoplasmic reticulum similar to that of neurons. The processes of these cells contain filaments. The distinct granules apparent within their cytoplasm

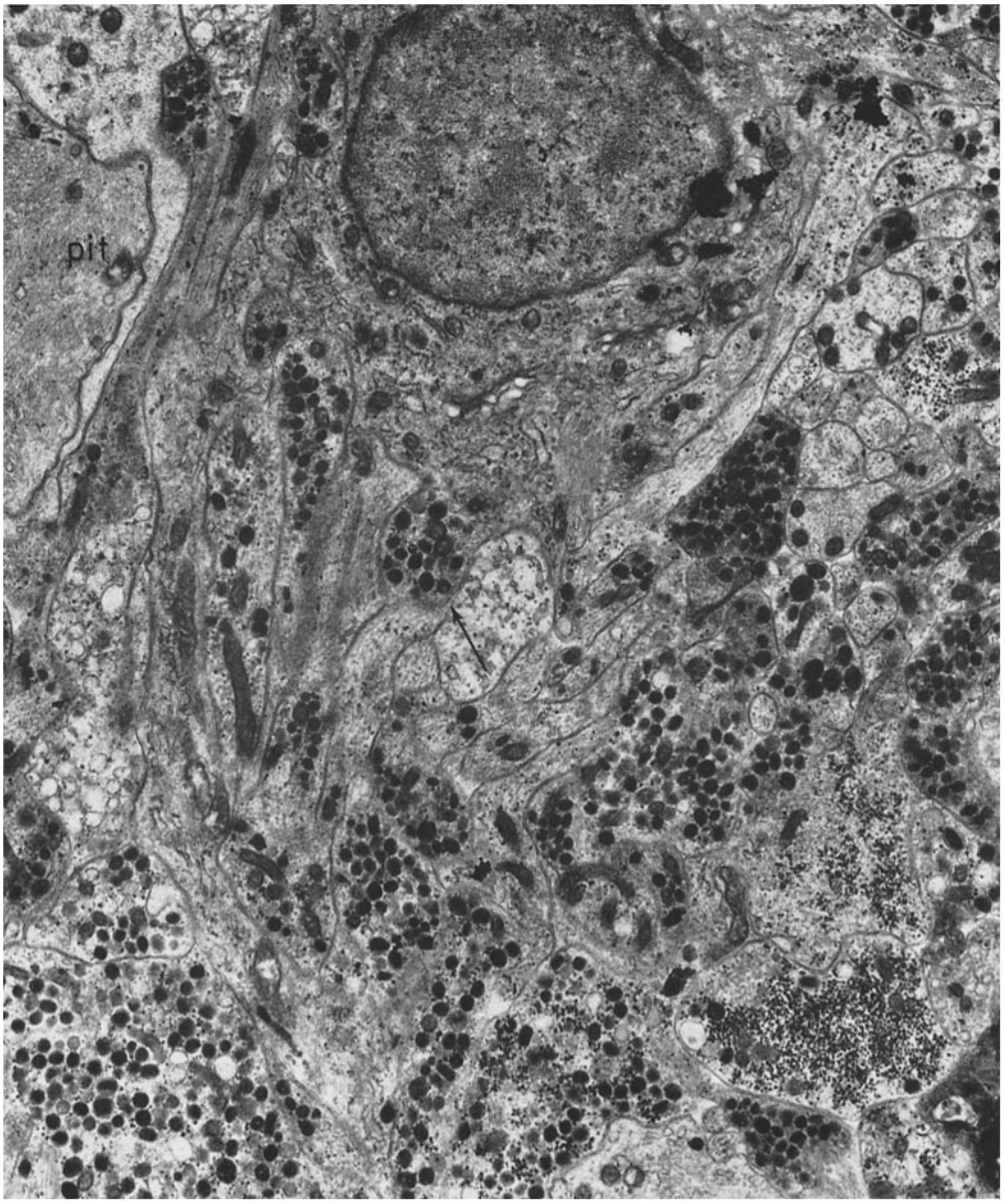

FIGURE 12 This section is through the hilar zone of the opossum neurohypophysis. Axons filled with neurosecretory granules and variable amounts of glycogen fill the lower part of the field. A single cell occupies the upper part of the field. This cell is identified as a neuron by its RNA-rich cytoplasm containing scattered filaments, and by its processes, some of which are filled with neurosecretory granules (arrow). A part of the cytoplasm of a pituicyte is evident (*pit*) and is filled with a dense feltwork of fibrils. Lead stained. × 5,000.

and processes (Fig. 12) are identical to neurosecretory granules in axons elsewhere in the neurohypophysis.

PITUICYTES: These are interspersed between axons in the hilar zone (Fig. 13). In the opossum these cells are large with round, ovoid, or irregularly shaped nuclei containing finely stippled chromatin. Occasional inclusions within the

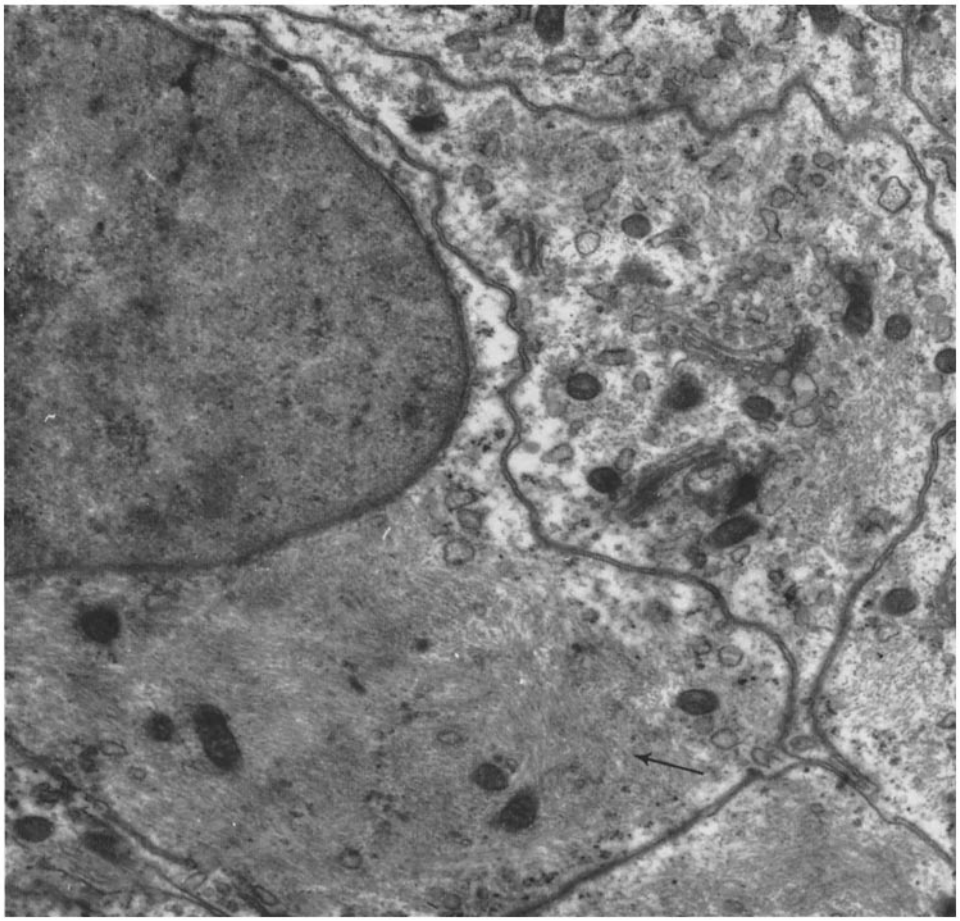

FIGURE 13 This micrograph demonstrates the usual appearance of the pituicyte in the opossum neurohypophysis. The nucleus is at the left. The cytoplasm is filled by a feltwork of fibrils (arrow). Lead stained. × 13,500.

nucleus are due to deep invaginations of cytoplasm similar to those frequently seen in neoplastic cells (19) (Fig. 14). Their cytoplasm contains numerous linearly arranged fibrils without periodicity that in cross-section appear as a coarse stippling. Scattered lamellae of rough-surfaced endoplasmic reticulum studded with ribonucleoprotein granules are present. Mitochondria are scant. Elongated cytoplasmic processes extend from these cells into the palisade zone.

THE SEPTAL REGION, separated from the parenchyma by a distinct basement membrane, contains fibroblasts, collagen fibers in cross- and longitudinal array, and capillaries. Endothelial cytoplasm is fenestrated as has been described in the rat by Palay (20) and by Hartmann (21). Apparent islands of axons within this extracellular space of the septal zone probably represent tangentially sectioned protrusions of parenchyma, since in favorable sections basement membrane outlines these islands. Similar structures also have been observed in the rat by Hartmann (21).

DISCUSSION

The lobularly organized neurohypophysis of *Didelphis virginiana* has a fine structure that differs from that of species previously studied. Its bulbous nerve terminals ensheathed by pituicyte processes form linear palisades at right angles to the border of the lobule. The structure of the nerve ending

itself is similar to that in other species. The pituicytes also differ from those previously described in that they have a densely fibrillar cytoplasm. They are in striking contrast to rat pituicytes that have a pale cytoplasm with numerous lipid inclusions (21), nor do they resemble the three types of rabbit pituicytes described by Fujita and Hartmann (22). Although the pituicytes of various animals show considerable species variation, all bear some resemblance to glial cells, either functionally or structurally. The presence of inclusions (of invaginated cytoplasm) (Fig. 14) within the nuclei of pituicytes corresponds to the observation of Bargmann (6) in light microscope studies on the opossum neurohypophysis.

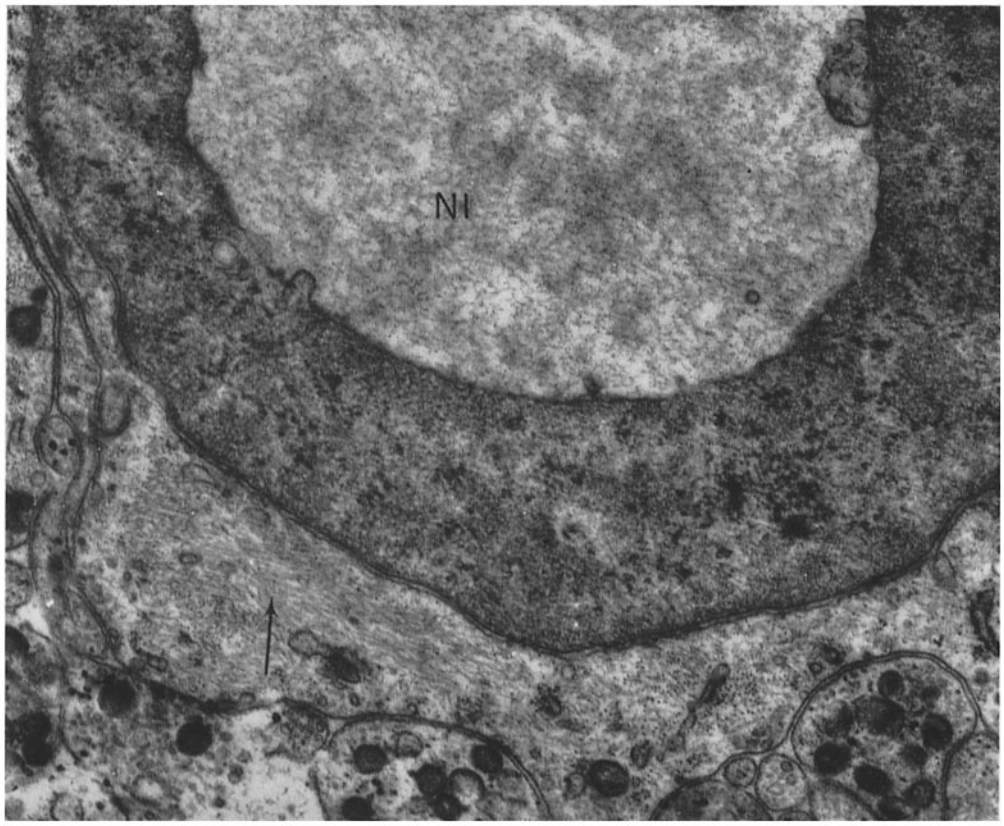

FIGURE 14 Electron micrograph of a pituicyte. Its fibril-filled cytoplasm is evident (arrow). The nucleus contains a large inclusion of invaginated cytoplasm (*NI*). Lead stained. × 20,000.

The rare occurrence within the neurohypophysis of cells that appear morphologically to be neurosecretory is unexpected (Fig. 12). However, nerve cells have been observed within the neurohypophysis of the porpoise by Geiling, Vos, and Oldham (23). Oldham also has reported neurons in the neurohypophysis of the armadillo (24), and Shanklin has seen them in the dog (25), chick (26), and human (27). Neurons have not been demonstrated previously in the opossum neurohypophysis by light microscopy. The one cell identified as a neuron in our electron micrographs is relatively small, so that it might be difficult to recognize by light microscopy. However, the dense ergastoplasm, prominent Golgi membranes, intracytoplasmic filaments, and neurosecretory granules within processes that resemble other neuronal processes lead us to believe that this cell should be considered a neurosecretory neuron.

Herring bodies usually have been identified by

their large size, dense packing of neurosecretory granules, and by comparison in size with the bodies that stain with chrome-alum-hematoxylin in light microscopic preparations. The large bodies noted in the present study correspond in size and location with the aggregates of stainable neurosecretory material present in the hilar zone of the opossum neurohypophysis when examined by light microscopy. Other somewhat smaller bodies with the same dense packing of neurosecretory granules also are observed and may be considered to be either Herring bodies or bulbous axons. In addition, occasional axons of normal caliber also are packed with neurosecretory granules. Thus, although Herring bodies seem to be a distinct entity, in both light and electron microscopy, intermediate forms between axons packed with neurosecretory granules and Herring bodies exist.

Glycogen-like material apparently has not been described previously within the axons of the neurohypophysis. It is present as both individual particles and aggregates that are correlated with the PAS-positive material removed by diastase digestion.

Essentially, then, the morphologic findings of Bodian (3) on the opossum neurohypophysis are confirmed. The electron microscopic observations are similar to those previously reported, with several important exceptions. The parenchyma is arranged in a definite pattern bounded by an extracellular space containing connective tissue and capillaries. A material that morphologically and histochemically is identical with glycogen has been demonstrated within axons. Rare neurosecretory nerve cells have been observed in the neurohypophysis of the opossum. Its pituicytes differ from those of species thus far described in that they are filled with intracytoplasmic fibrils.

This work was supported in part by grants from the United States Public Health Service (B-425, RG-3784, 2G-240, and 2G-897), and from the Beaumont-May Institute of Neurology, Washington University. Dr. Roth is a trainee in Experimental Pathology.

We are indebted to Mrs. Lois Grawe, Miss Betty Smallfelt, and Mrs. Ann Dyson for their technical assistance.

Received for publication, May 31, 1963

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