PLURIVESICULAR SECRETORY PROCESSES AND NERVE ENDINGS IN THE PINEAL GLAND OF THE RAT

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ABSTRACT

The pineal body of white normal rats, 1.5 to 3 months old, was studied under the electron microscope. A single type of parenchymal cell—the *pinealocyte*—is recognized as the main component of the tissue, and some of the structural characteristics of the nucleus and cytoplasm are described. The main morphological characteristic of the pinealocytes is represented by club-shaped perivascular expansions connected to the cell by thin pedicles. They are found lying in a large, clear space surrounding the blood capillaries. The name *plurivesicular secretory processes* is proposed, to emphasize the main structural feature and the probable function of these cellular expansions. A tubulofibrillar component is mainly found in the pedicle, and within the expansion there are numerous small mitochondria and densily packed vesicles of about 425 A. Two types of vesicles, one with a homogeneous content and another with a very dense osmium deposit, are described. Between the two types there are intermediary forms. In these processes, mitochondria show profound changes which may lead to complete vacuolization. The significance of this plurivesicular secretory component is discussed in the light of recent work on the biogenic amines of the pineal body and preliminary experiments showing the release of the vesicles containing dense granules after treatment with reserpine. These vesicles are interpreted as the site of storage of some of the biogenic amines. Bundles of unmyelinated nerve fibers and endings on large blood vessels which also contain a plurivesicular content are described and tentatively interpreted as adrenergic nerve terminals.

Although the pineal gland has been known anatomically since ancient times, the knowledge of its structure and function has progressed very little. The enormous published literature on the histology and innervation of the gland, as well as on its correlation with other endocrines and its role in metabolism, has been so far rather negative and confusing (see 1, 2).

In recent years the interest in this tissue has increased considerably because of the work of Farrell (3, 4), which tends to indicate the presence of an adrenoglomerulotrophic factor in pineal extracts capable of increasing aldosterone secretion.

Other regions of the midbrain, including the area surrounding the aqueduct and the subcommissural organ, would seem to be also involved in aldosterone control. The situation has become more complex with the postulation of an inhibitory factor of aldosterone also derived from the pineal gland (5).

Of particular significance are the observations of Giarman *et al.* (6–8) of a high concentration of biogenic amines in the pineal body. Histamine, catechol amines, and 5-hydroxytryptamine are all exceptionally concentrated in this tissue. Giarman *et al.* (8) have found that the serotonin levels in human and simian glands are the highest ever reported for any neural structure of any species so far examined.

In addition to these amines there is the pineal hormone, melatonin, described by Lerner *et al.* (9). This substance which antagonizes the skin-darkening effect of the melanocyte-stimulating hormone is chemically a 5-methoxy-*N*-acetyltryptamine, and probably derives from 5-hydroxytryptamine by *O*-methylation and *N*-acetylation. Recently, Axelrod (10) has found that the two enzymes involved in these chemical transformations are found in the pineal gland.

The best histological information comes from the work of del Río Hortega (11–13), who ended previous discussions on the cell types by demonstrating that the pineal consists essentially of a single, specific cell type, named *pinealocyte*, which is different from the glia or the neurons, although of the same nervous origin. Morphologically the pinealocyte is characterized by an irregular contour with several thin processes ending in a club-shaped expansion in the perilobular or perivascular spaces. At variance with the astrocytic vascular feet, these pinealocytic expansions do not attach to the capillary wall but end in the subendothelial connective tissue (11). So far the attempts to demonstrate the secretory nature of this tissue at the level of the optical microscope have failed or are not convincing.

The problem of innervation of the pineal body is also debated. According to Cajal (14), it receives exclusively sympathetic perivascular nerve fibers. This view, which excludes the possible central control of the gland, is supported by Herring (15) for the rat. On the contrary, Ikuta (16), in experiments on pinealectomy in the rat, finds experimental evidence of a nerve tract between this body and the habenula. The same type of investigation in the cat gave negative results (17). Gardner (18), in the hooded rat, finds nerve fibers of central and of sympathetic origin, the former coming mainly by way of the habenular commissure and ending among the pinealocytes, away from the blood capillaries. The sympathetic fibers, exclusively unmyelinated, accompany and innervate the pial blood vessels penetrating into the gland (18).

Kappers (19), in an extensive study of the development and innervation of the epiphysis of the rat, gives conclusive evidence that the innervation is autonomic and supplied bilaterally by the superior cervical ganglia. Extirpation of these sympathetic ganglia produces degeneration of the innervating fibers. The author reaches the conclusion that the innervation is mainly, if not exclusively, orthosympathetic, and that "neither nervous nor vascular relations have been observed pointing to the existence of an epithalamo-epiphyseal or habenulo-epiphyseal complex that, in any way, could be compared with the hypothalamo-hypophyseal complex."

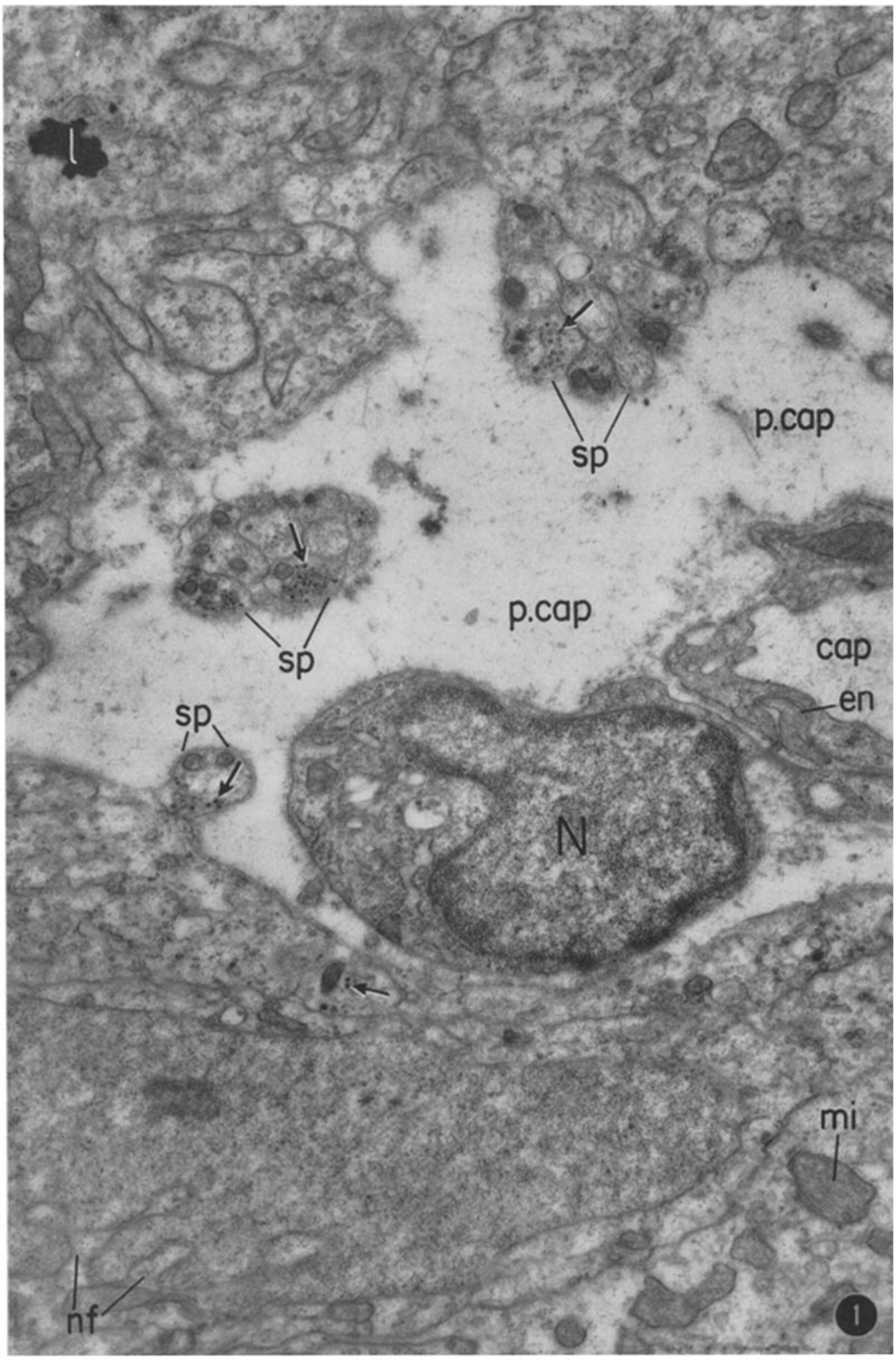
Our knowledge of the submicroscopic mor

Abbreviations Used in the Figures

Ax, axon
bm, basement membrane
cap, capillary
dmi, degenerating mitochondrion
dv, dense vesicle
en, endothelium
er, erythrocyte
f, tubular fibrillar elements
l, lipid droplet
mc, muscle cell
mi, mitochondrion
N, nucleus
nf, nuclear folding
nu, nucleolus
p, pore
p.cap, pericapillary space
pe, pedicle
sp, secretory process
sv, synaptic vesicle
v, vesicular material

FIGURE 1

Low power electron micrograph of the pineal gland of the rat showing a large pericapillary space (*p.cap.*). At the bottom, a pinealocyte with the nucleus showing nuclear infolding (*nf*) and the nucleolus. Several plurivesicular secretory expansions (*sp*) are seen in the pericapillary space. Some of them are cut through the pedicle and show a fibrillar component. The arrows indicate some of the vesicles containing a dense granule. × 18,500.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phology of the gland is just beginning. Milofsky (20, 21) describes the presence of a vesicular material with dense granules near the blood vessels. The author, stressing the similarity of this tissue to the neurohypophysis, interprets this component as belonging to nerve endings of unknown origin. In a recent demonstration abstract on the pineal of the cow, Anderson (22) describes the parenchymal cells of the pineal as star-shaped elements with perivascular processes filled with mitochondria.

In the present paper some of the most prominent ultrastructural characteristics of the pinealocyte will be described and its secretory nature recognized. A plurivesicular structure consisting of homogeneous vesicles and other vesicles containing a dense osmium deposit will be shown to be the main characteristic of the perivascular secretory processes. Nerve endings which contain a morphologically similar plurivesicular structure and are found near the large blood vessels will be described and interpreted tentatively as adrenergic endings.

The suggestion will be made that the secretory processes probably contain the biogenic amines that are synthesized and secreted by the pineal gland.

TECHNIQUES

Normal white rats, 1.5 to 3 months old, were anesthetized under ether and the pineal body was dissected out, cut into small pieces, and fixed in cold 1 per cent OsO_4 in Periston Bayer at pH 7.2–7.4 (23) for 2 hours. The embedding was done in a 9:1 mixture of butyl and methyl methacrylate and the sections of silver or gray color were cut with a Porter-Blum microtome. A Siemens Elmiskop I and a RCA EMU 2E electron microscope with a compensated objective lens and with a condenser aperture of 250 μ and an objective aperture of 50 μ were used.

OBSERVATIONS

1. *Submicroscopic Morphology of the Pinealocyte*

Essentially one type of parenchymal cell—the pinealocyte—can be recognized in the pineal gland of the rat. The cells are polygonal in shape and tightly packed into thick trabeculae without definite intercellular clefts. This compact aspect is in contrast with the looser packing of the cells revealed with the silver staining techniques in Río Hortega's (11) illustrations. In between these cellular trabeculae there are large spaces, in the center of which blood capillaries are present. The wide perivascular spaces are notably clear and contain the secretory processes of the pinealocytes (Figs. 1 and 7).

Pineal cells have a rather polymorphous nucleus with numerous and deep infoldings of the nuclear membrane (Figs. 1 and 2). These infoldings contain portions of cytoplasm with mitochondria and even lipid inclusions. This fact may give rise to faulty interpretations at the level of the optical microscope, such as the description of intranuclear granules (see 11). A nucleolus is frequently observed in the sections (Fig. 1). The cytoplasm is rather scarce and contains elongated and irregularly shaped mitochondria. These mitochondria have the typical structure with the double membrane and the inner crests. They become smaller and denser near the edge adjacent to the perivascular space and also inside the cell processes (Fig. 1).

A few irregularly shaped lipid droplets of the size of mitochondria or somewhat larger are constantly found in the pinealocyte.

In the hyaloplasm there are groups of dense, free ribosomes and a few membranes of the endoplasmic reticulum; medium sized, clear vacuoles are also observed. At the periphery of the cell and in some cell processes these vacuoles become more abundant (Fig. 1). In addition there may be found small vesicles with a light, homogeneous content and others containing a dense granule of osmium in the center (Figs. 3 and 4). These two types of vesicular elements are preferentially located in the secretory processes of the pinealocyte and will be described subsequently.

2. *Secretory Processes of the Pinealocyte*

Our observations on the rat are in line with Río Hortega's observations (11), in other mammals, of the club-shaped perivascular expansions of the pineal cells. In thin sections for the electron microscope it is often difficult to find the connections of these enlarged processes with the cell edge. This is because the pedicles are rather thin and of variable length. In Fig. 1 several pedicles sectioned at different angles are shown. They contain, in addition to a few mitochondria and some vesicles, an elongated component made of fine tubular elements similar to the neuroprotofibrils found in nerve axons. These fine

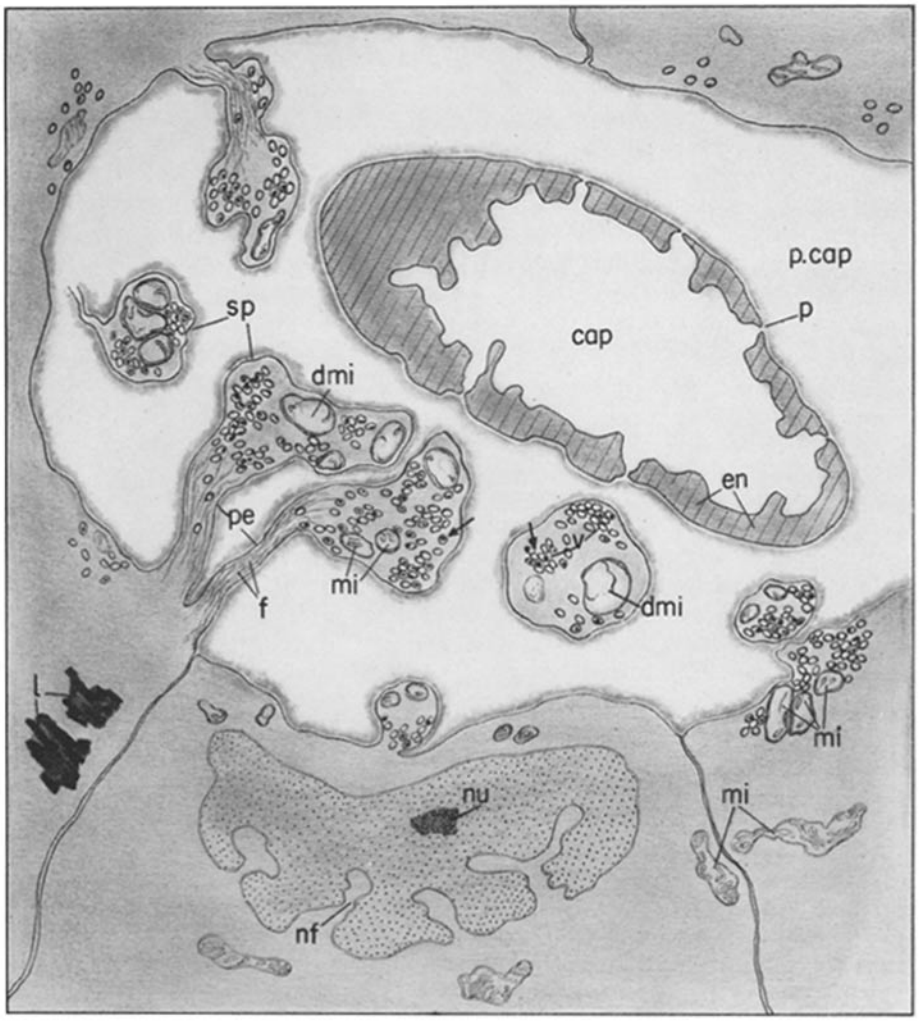


FIGURE 2

Diagram of a pericapillary region of the pineal gland of a young rat. Some plurivesicular secretory expansions (*sp*) containing mitochondria (*mi*) and the two types of vesicles are shown. The pedicles (*pe*) connecting the secretory processes with the cell contain tubular fibrillar elements (*f*). The capillary (*cap*) shows an endothelium (*en*) with pores (*p*). Arrows point to vesicles containing a dense granule. See further description in the text and legends.

tubules are often found within the cytoplasm proper of the pinealocyte, but they tend to converge and penetrate into the pedicle of the cell process.

The enlarged portion of the secretory process contains several small and dense mitochondria, some of which show alterations of the inner structure with loss of the crests and vacuolization, the large vacuoles still retaining the double mitochondrial membrane (Figs. 3, 4, and 6).

The main component that occupies the expansion is made up of round or oval vesicles with a mean diameter of 425 A and having a definite limiting membrane. These elements resemble the synaptic vesicles (24) and have about the same size distribution (Fig. 5). However, a striking difference is that there are two types of vesicles, according to their content, with probable intermediary forms. Most of the vesicles have a homogeneous content, but interspersed among

them are others which have a very dense, round osmium deposit with a mean diameter of 210 A (Fig. 5). These characteristics explain the name *plurivesicular secretory processes* coined for these cellular expansions. These vesicles, with or without dense granules, are similar to those observed occasionally within the cytoplasm of the pinealocyte (Figs. 1, 3, and 4), but in the cell processes they are much more abundant and more densely packed.

That the secretory processes are indeed connected to the cell and that they do not belong to nerve endings, as assumed by Milofsky (20, 21), is clearly observed in Figs. 3 and 4.

In Fig. 3, several finger-like expansions extend from the cytoplasm of the pinealocyte (on the left side) into the pericapillary space. The diameter of the pedicles is 0.2 to 0.25 μ. Fig. 4 shows a more unusual case of a large expansion connected to the pinealocyte by a thicker pedicle. In both figures there are a few heterogeneous vesicles with dense osmium deposits (marked with arrows) within the cytoplasm. Within the secretory processes the plurivesicular material is abundant and the mitochondria show degenerative changes.

A more detailed view of the homogeneous and the heterogeneous vesicles is observed at higher magnification in Figs. 6 and 7. In the latter, a club-shaped expansion is seen below the endothelium of a blood capillary. In this case there is a thin pedicle, and a large dense droplet (probably lipid) within the expansion. This lipid material is similar to that found within the pericaryon of the pinealocytes and has never been observed in nerve axons.

At the perivascular spaces mentioned above, there is an amorphous coating that covers the edge of the cells and cell processes in addition to the endothelial elements of the capillary. In adult animals collagen fibrils can be observed within this space.

The capillaries have a discontinuous endothelium perforated with pores of 800 to 1600 A (Fig. 7). The lumen of the capillaries is often reduced to a thin slit because of the bulging of the nuclear portion of the endothelial cell.

3. *Preliminary Observations on Nerve Fibers and Endings in the Pineal Gland*

Occasionally nerve fibers have been observed in sections of the pineal gland. A bundle of mostly unmyelinated nerve fibers but with a few myelinated ones was found near the capsule of the pineal body. More frequently, small bundles of unmyelinated fibers contained within a Schwann cell have been found in the trajectory of the larger vessels entering the gland. Smaller, free axons reaching the muscle cells of a blood vessel can be observed in Fig. 8. The axons show the well known structure with mitochondria and fibrillar elements, but near the endings they contain groups of vesicles similar in size and shape to the synaptic vesicles found in synaptic endings. A few of these vesicles contain a dense granule inside, as in the case of the secretory processes of the pinealocyte.

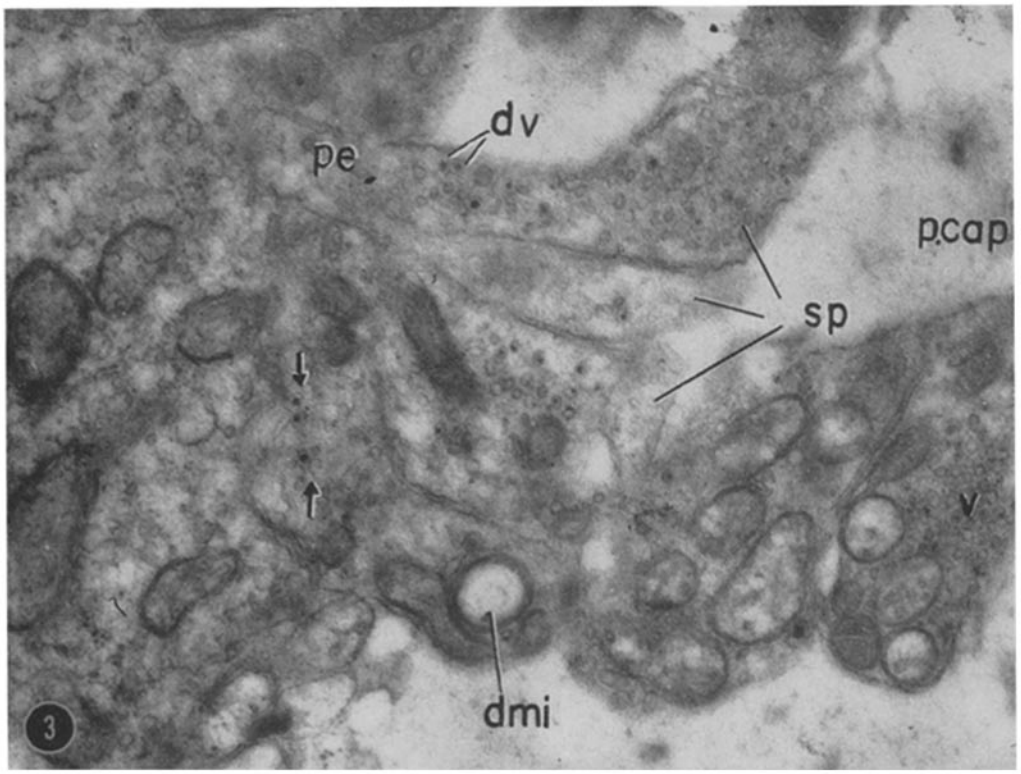
Fig. 9 shows another nerve bundle, within the pineal gland, in which some of the axons contain homogeneous and heterogeneous vesicles. This vesicular material, which is strikingly similar in morphology to that found in the expansions of

FIGURE 3

Electron micrograph of material similar to that of Fig. 1. Toward the left, the apical portion of a pinealocyte with several mitochondria and finger-like secretory processes (*sp*) projecting into the pericapillary space (*p.cap*). The continuity of these processes with the cell is clearly seen. The arrows mark some heterogeneous vesicles containing a dense material. The secretory expansions are filled with plurivesicular material and mitochondria. × 40,000.

FIGURE 4

Description similar to that of Fig. 3. One of the secretory expansions (*sp*) shown in this figure is unusually thick, and the connection with the cell is clearly seen. The arrows point to some dense vesicles situated deep in the cytoplasm. The expansion contains abundant plurivesicular material and degenerating mitochondria. × 45,000.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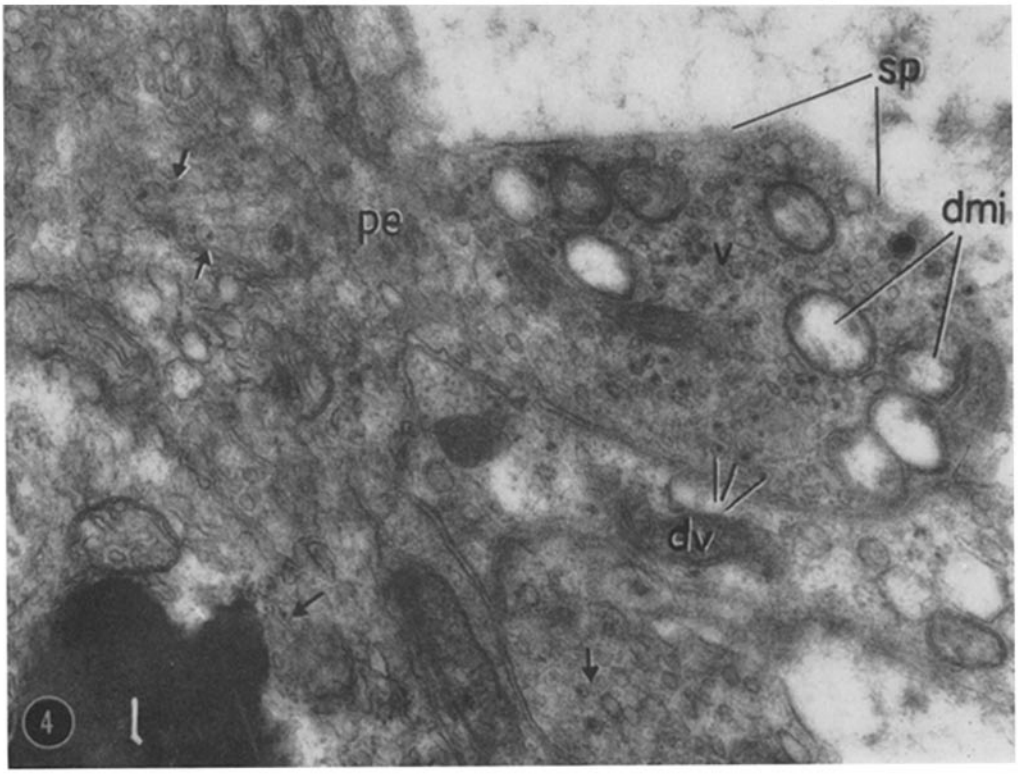
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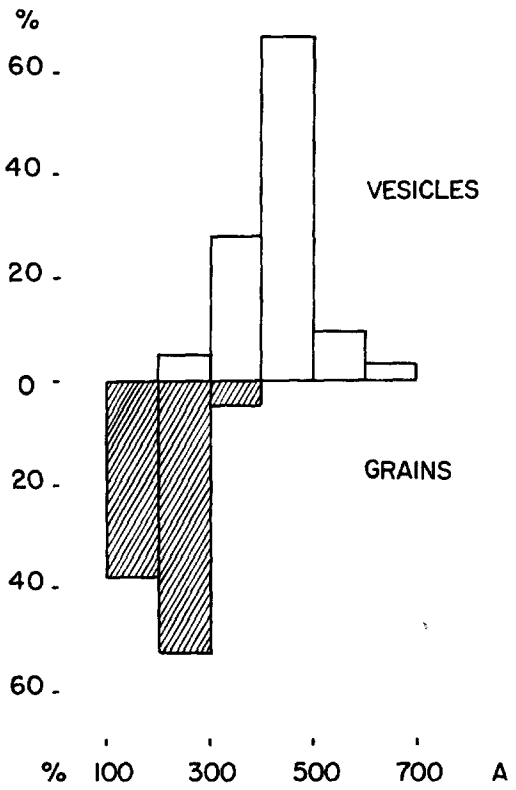


FIGURE 5

Histogram indicating the percentage of different sizes (in A) of 200 vesicles and 100 grains.

the pinealocyte, has also been recently described in other adrenergic nerves by De Robertis and Pellegrino de Iraldi (25).

DISCUSSION

The observations reported here offer definite evidence that pinealocytes are secretory cells. The secretion represented by the small plurivesicular material is produced and accumulated preferentially within the perivascular expansions of the cell.

The morphological interpretation of these secretory processes (Fig. 2) is in line with the early observations of Río Hortega (11), with the light microscope, on other mammals. In his preliminary electron microscope study Anderson (22) also supports this viewpoint, whereas Milofsky (20, 21) interprets the processes as nerve endings.

The difficulty of observing in each case the cellular connection of the process is explained by the thin, and sometimes long, pedicle involved. The components present in the pedicle—thin tubules—and in the club-shaped expansion—mitochondria and plurivesicular material—can also be found, although with less frequency, in the cytoplasm of the pinealocyte. Our interpretation is further stressed by comparing the cell expansions (Figs. 1, 3, and 4) with the typical unmyelinated nerve fiber ending on the larger blood vessels of the pineal (Figs. 8 and 9).

The plurivesicular material of the secretory expansion should be interpreted in relation to the recent knowledge of the high concentration of biogenic amines in the pineal gland (6–9) and the fact that several of these amines, particularly the catechol amines and serotonin, have an intense reducing effect on osmium tetroxide. This histochemical reaction has been widely used in the adrenal medulla at the level of both the optical and the electron microscope (26–28). Thus the catechol secretion droplets can be followed throughout the process of formation, storage, and secretion because of their extraordinarily high electron density (29).

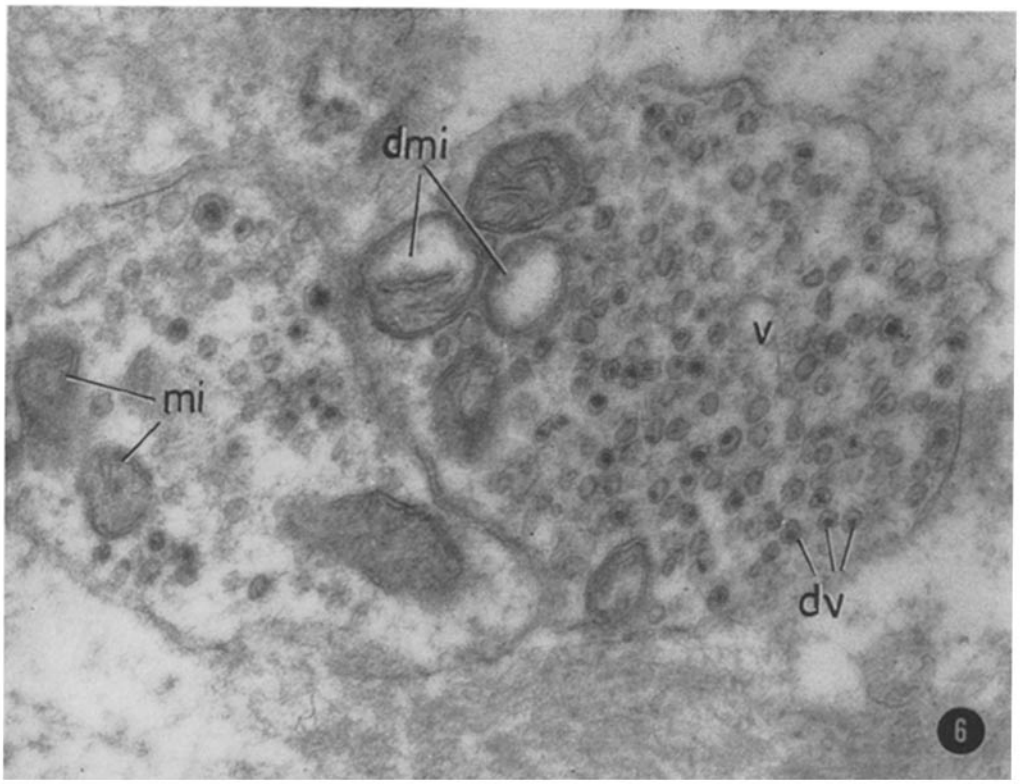
The dense granules observed within some of the vesicular components of the pineal could also

FIGURE 6

Electron micrograph of the normal pineal gland of the rat showing two secretory processes at high magnification. Note the characteristic plurivesicular material with clear and dense vesicles. × 80,000.

FIGURE 7

Similar to Fig. 6. One secretory process with the thin pedicle (*pe*) and the club-shaped enlargement filled with plurivesicular material is located below the endothelium (*en*) of a blood capillary. Notice the presence of a lipid droplet (*l*) in the process similar to those found in the cytoplasm of the pinealocyte. × 100,000.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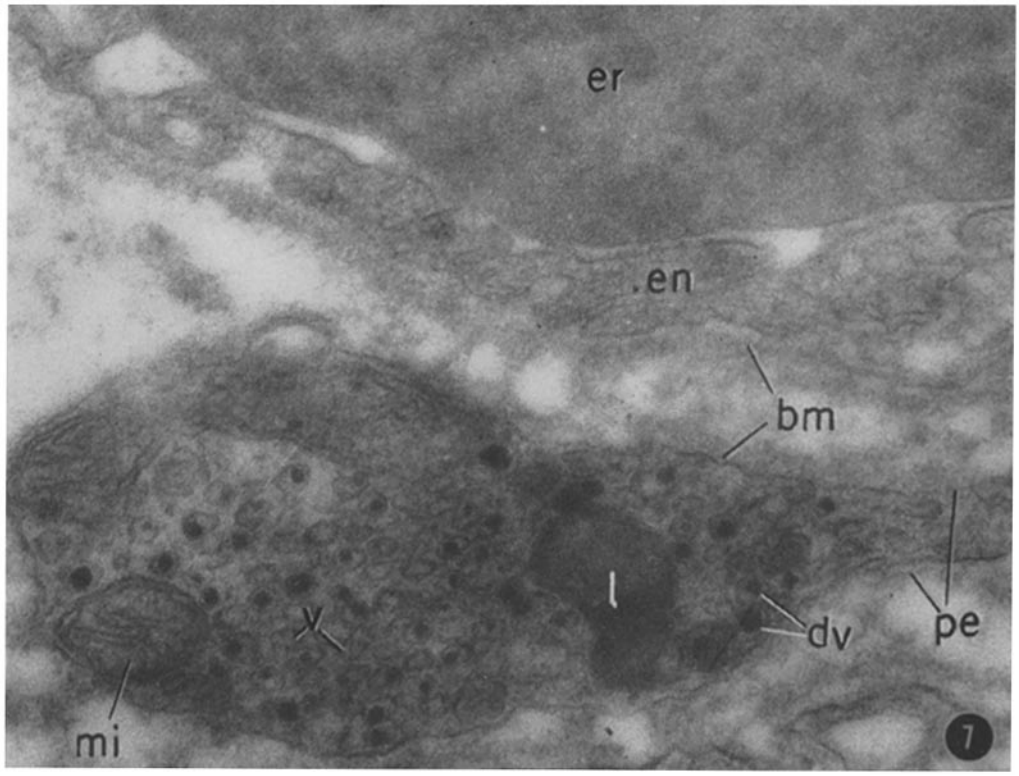
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indicate the presence of reducing amines within the vesicles. The electron microscope image of this plurivesicular component of the pineal is very similar to that of the material found occasionally by De Robertis and Vaz Ferreira (30) in some adrenal glands of the rabbit after stimulation of the splanchnic nerve. This component was also interpreted as representing catechol amines.

Our interpretation of the dense granules and vesicles is strongly supported by preliminary experiments showing the rapid disappearance of all dense granules after a single injection of reserpine, followed by restoration after a few days (31). It is well known that this drug induces an almost complete release of biogenic amines from the nervous tissue (32). The presence of numerous mitochondria in the cell expansion, some of which show profound changes, indicates that they may be related in some way to the formation or release of the secretion, but at present there is no indication as to what mechanism may be involved.

The synthesis of complex biogenic amines such as melatonin (10) involves the interplay of several enzymes whose exact intracellular location is barely known (9). It is conceivable that our finding of a specific submicroscopic structure for the pinealocyte may lead to a better understanding of the mechanism of synthesis, release, and inactivation of biogenic amines in the pineal gland.

The observations on the innervation of the gland are still preliminary. Several data in the literature indicate that the nerve fibers innervating the pineal gland are of sympathetic origin. Some of these nerve fibers and endings contain a plurivesicular material which is morphologically similar to that found in the secretory expansions of the pinealocytes. The presence of dense granules within some of the vesicles at the nerve endings may presumably indicate the presence of reducing amines such as norepinephrin. A similar component has been observed in the adrenergic splenic nerve (25). The finding of this plurivesicular content in adrenergic nerve terminals is interesting when this material is compared with the more homogeneous vesicular content of most synaptic endings (see 24). It also indicates that a similar microvesicular entity is involved in synapses and peripheral neuroeffectors.

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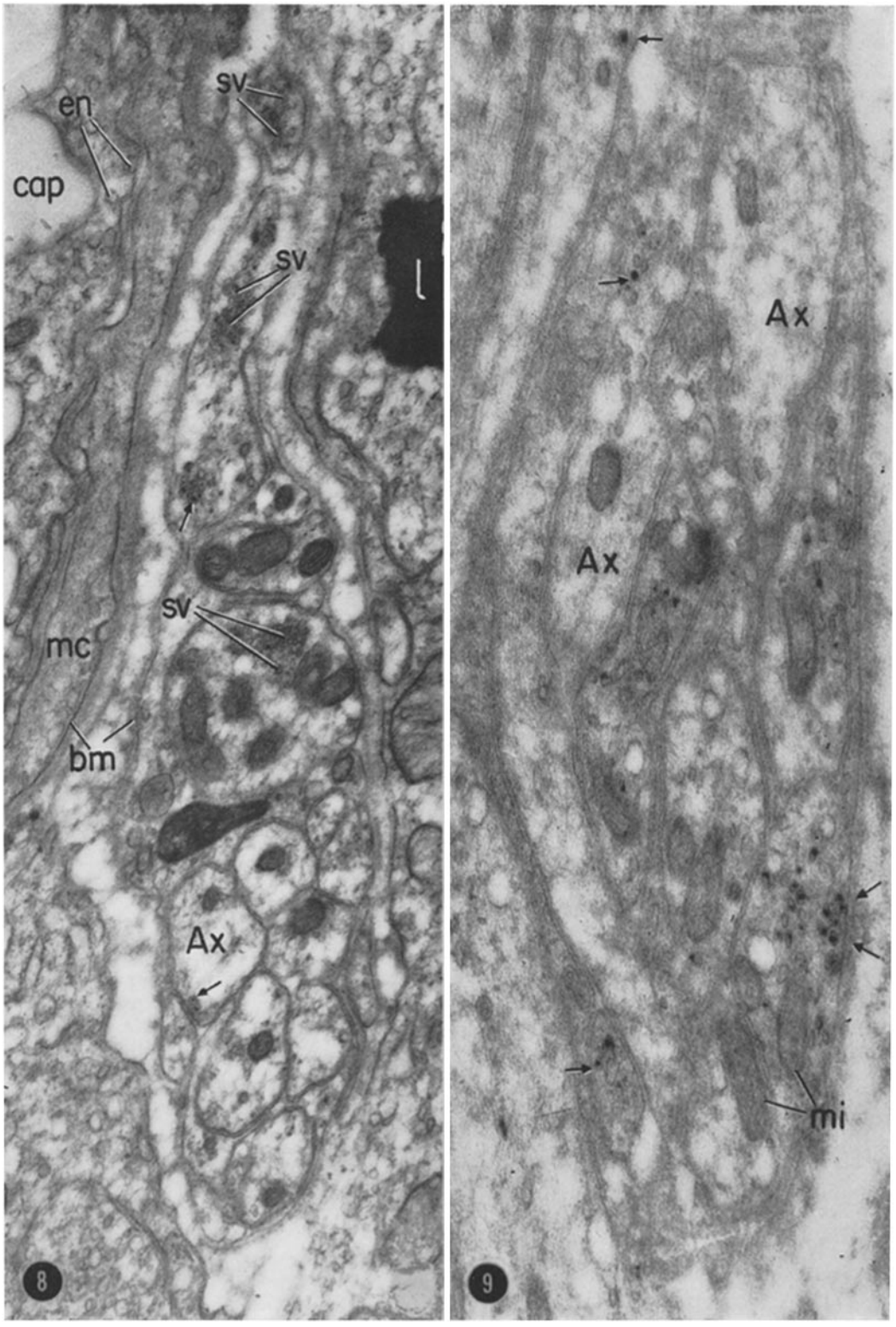
FIGURE 8

Unmyelinated nerve fibers abutting on a large blood vessel. Some of the endings show a plurivesicular material. The arrows indicate some of the vesicles containing a dense granule. × 28,000.

FIGURE 9

Electron micrograph showing a bundle of unmyelinated nerve fibers in the pineal gland. Within the axoplasm, in addition to neuroprotofibrils, mitochondria, and some clear vacuoles, there are clusters of homogeneous (clear) and heterogeneous vesicles. Some of the latter are marked with arrows. This plurivesicular material is characteristic of adrenergic nerves. × 46,000.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