MORPHOLOGIC EVIDENCE OF PHOTORECEPTOR DIFFERENTIATION OF PINEALOCYTES IN THE NEONATAL RAT

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ABSTRACT

The pineal body and the retina of the neonatal Sprague-Dawley rat were studied by light and electron microscopy, and the morphologic differentiation of the parenchymal cells of the pineal body was compared with that of the developing photoreceptor cells of the retina.

Between the ages of 4 and 12 days after birth, some of the developing pinealocytes were observed to become elongated and polarized, with their nuclei located at one pole. "Synaptic" ribbons were observed within the cell body. At the opposite pole the cells developed elongated cell processes that initially contained microtubules and ribosomes. These cell processes projected into luminal spaces and were attached by structures resembling zonulae adherentes to the adjacent cells. Extending from the tips of the cell processes, cilia with a 9 + 0 arrangement were observed. Lamellated and vesicular membranes were noted at the tips of the cilia. Such morphologic differentiation, however, could be observed only in rats younger than 17 days.

Comparison of the morphologic features of the neonatal pinealocytes with those of the developing retinal photoreceptor cells showed much similarity. It is suggested that the pinealocytes of the neonatal rat undergo "photoreceptor-like" differentiation during a transient neonatal period. Such morphologic differentiation may provide an explanation for light-induced biochemical changes described in neonatal rats whose eyes had been enucleated.

It has been shown that the pineal organs of various submammalian vertebrates contain functional photoreceptor elements, the morphologic features of which are very similar to those characteristic of retinal photoreceptors (1, 2). These structures are not apparent in the adult mammalian pineal organ, which displays cytologic and histologic features characteristic of a solid glandular organ (3, 4). Studies of this phylogenetic development of the pineal from a photoreceptor organ to a secretory gland (5) have led to the proposed theory that the pinealocytes of mammals belong to a sensory cell line that has evolved from the sensory pineal photoreceptor of lower vertebrates (6).

This theory of transformation from photoreceptor cell to a secretory pinealocyte during phylogenetic development allows for the existence of intermediate cell types found in the pineals of chelonians, lacertilians, and birds. These are designated as "rudimentary photoreceptor cells" (6) or "pseudosensory cells" (7, 8).

Furthermore, various predominant cell types of this sensory cell line have been identified during different stages of development in the life cycle of some species. This is exemplified by the ontogenic development of the pineal organ in the newt *Taricha torosa*. During the early larval stages, typical pineal photoreceptor cells are predominant. After metamorphosis, however, the outer segments of the photoreceptor cells are mostly disorganized and detached, and the organ develops morphologic features suggestive of a secretory organ (9–11).

The cytologic features of pinealocytes of the adult rat are consistent with a secretory organ (12). The secretory activity is regulated by environmental lighting (13–15), a control which operates in an indirect manner by way of the optic pathways and the sympathetic fibers that innervate the pineal (16). Although the pinealocyte in the adult is believed to have lost the capacity for direct photoreception, the possibility has been suggested that the pineal organ of the neonatal rat may be a photosensitive structure (17, 18). Recent studies of fetal rats show that pinealocytes "exhibit surface modifications reminiscent of developing photoreceptor cells" (19, 20). In the neonatal rat, biochemical responses to light were demonstrated in the pineal after enucleation of both eyes (17, 21). These responses could be prevented by placing opaque hoods over the heads of the animals. Similar responses cannot be observed in adult rats. This evidence suggests the presence of an extraocular photoreceptor in the head, the location of which has been speculated but not yet defined (22, 23). The present study was undertaken to examine the pinealocytes of neonatal rats for morphologic evidence suggestive of developing photoreceptor cells.

MATERIALS AND METHODS

The pineal bodies and retinas of 24 Sprague-Dawley rats were studied by light and electron microscopy. The rats were maintained at room temperature under diurnal lighting conditions (12-h cycle) and sacrificed after birth at the following intervals: two animals each at 1, 4, 9, 13, 15, and 17 days; four at 11 days; five at 12 days; one at 22 days; and two at 96 days. The sex of the animals was not considered in this study. The pineal bodies were removed while the animals were under anesthesia with methoxyflurane. A skull flap with adherent meninges and pineal body was taken from each animal and immediately immersed in cold 4% phosphate-buffered glutaraldehyde. While the tissues were immersed in the fixative, the pineal bodies were dissected from the meninges and calvaria and fixed for a minimum of 2 h in fresh fixative. The tissues were then rinsed in Sorensen's phosphate buffer, postfixed in Dalton's chrome-osmium fixative, dehydrated in ascending concentrations of ethanol, and embedded in epoxy resin. The eyes were enucleated, and the retinal tissues were fixed and embedded in the same fashion. Sections were then cut at a thickness of 1–2 μm and stained with toluidine blue for light microscope study. Thin sections for examination by electron microscopy were doubly stained with uranyl acetate and lead citrate.

RESULTS

During the first 17 neonatal days, the pineal body changed remarkably in size and shape (Fig. 1). At 1 day of age, the configuration was that of an elongated ellipsoid that measured approximately $300 \times 300 \times 750\ \mu m$. By the 17th day, the area in the midcoronal plane of section had increased sevenfold, and the shape was spheroidal and bulbous.

These gross changes coincided with the vascularization of the organ and with active proliferation and organization of the pineal cells. At 1 day of age, the pineal body was composed of a dense cluster of randomly arranged parenchymal cells surrounded by connective tissue and blood vessels. Infolding of these connective and vascular tissues began on the first postnatal day. By 4 days of age, the peripheral regions of the organ were infiltrated by connective tissue and vascular channels, while the central portion consisted largely of parenchymal tissue (Fig. 1 *b*). Mitotic figures were observed frequently during these first 4 days of development (Fig. 2). They were observed with decreasing frequency until last noted at 15 days. During the first 4 days, the pinealocytes contained ovoid nuclei with prominent nucleoli, and, in some cells, invaginations were observed in the nuclear membrane (Fig. 3 *a*). The cytoplasm contained microtubules, mitochondria, rough-surfaced endoplasmic reticulum, free ribosomes, glycogen, and occasional lipoidal bodies. Some of these pinealocytes displayed well-formed "synaptic" ribbons[1] surrounded by "synaptic" vesicles (Fig. 3 *d*). Cilia of both 9 + 0 and 9 + 2 patterns were present at this time period (Fig. 3 *b*, *c*).

[1] In view of recent evidence suggesting a functional role on the part of these organelles in mammalian pinealocytes (24–26), the term "synaptic" ribbon is used rather than "vesicle-crowned rodlet" (27).

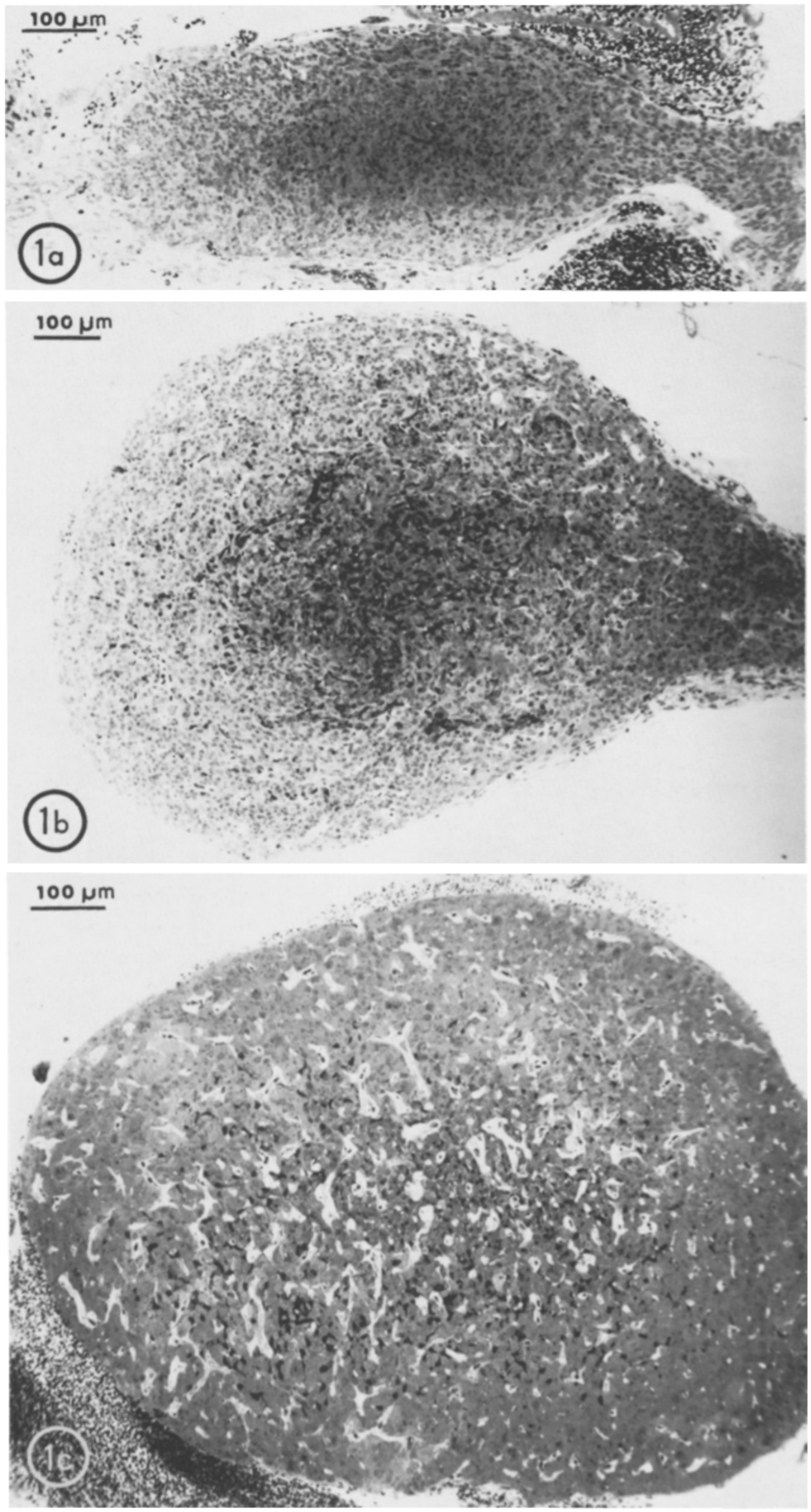


FIGURE 1 Light micrographs of the pineal body of the rat during postnatal development. Fig. 1 *a*, A midsagittal section at 1 day of age reveals a solid mass of undifferentiated pinealocytes without vascular connective tissue. Fig. 1 *b*, A midsagittal section at 4 days shows an increase in the overall dimensions resulting from cellular hyperplasia and hypertrophy. A capillary network penetrates the peripheral region. Fig. 1 *c*, A midcoronal section at 17 days reveals a dramatic increase in the size of the pineal body and thorough penetration of the parenchyma by vascular and connective tissue elements. *a*, × 100; *b*, × 90; *c*, × 100. AFIP neg. no. 72-12003-1.

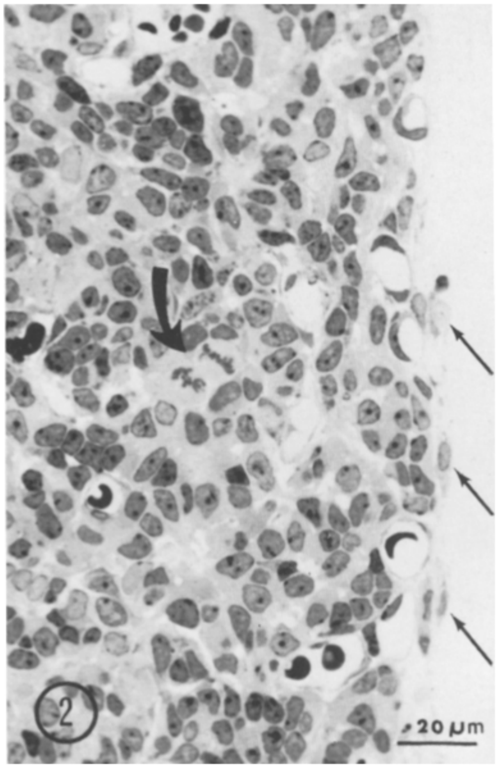


FIGURE 2 The peripheral region of the pineal body at 1 day of age. The surface connective tissue lies to the right (small arrows). A mitotic figure in late anaphase (large arrow) and numerous capillaries are observed within the randomly arranged undifferentiated pinealocytes. × 525. AFIP neg. no. 72-12003-2.

Between 4 and 12 days of age, the pinealocytes were organized into tenuous, cordlike formations that were surrounded by a connective and vascular tissue matrix (Fig. 4 *a*). Some of the cells were elongated and polarized, their nuclei at one pole and elongated cytoplasmic processes at the opposite pole (Fig. 4 *b*, *c*). The nuclei of these cells were indented and polymorphic, containing prominent nucleoli. Intranuclear filaments were noted in some instances. "Synaptic" ribbons were found near the nuclear pole of the cells (Fig. 5 *a*). The ribbons were observed mostly in groups of two to six. They were oriented perpendicular to the plasma membrane and formed arciform densities at their junctions with the plasma membrane (Fig. 5 *b*). There were no "postsynaptic" membrane thickenings or dense material within the "synaptic" cleft.

The elongated cytoplasmic processes of these pinealocytes contained numerous microtubules oriented parallel to the long axis of each cell. These processes formed dilated bulbous endings that contained free ribosomes, vesicles, rough endoplasmic reticulum, glycogen, mitochondria, and basal bodies (Figs. 6–8). Some of the pineal processes formed villi (Fig. 6 *b*). From the bulbous ends of other processes, cilia with nine peripheral filaments and no axial filament (9 + 0 pattern) were noted to develop (Figs. 9, 10). No cilia of 9 + 2 pattern were observed at this time period.

Adjacent to the tips of the cilia, membranous structures were observed. Some of these membranes consisted of vesicles confined by an outer plasma membrane (Fig. 11 *b*). Others consisted of whorls of membranes arranged in a lamellated fashion (Fig. 11 *a*, *c*).

The bulbous endings, cilia, and membranous structures were observed to extend into luminal spaces. These luminal spaces were observed first at the periphery of the pineal organ at 4 days of age and then centrally at 9 days of age. These lumina were located within cords of pinealocytes that separated them from the connective tissue spaces. The small, uniform size and distinctively round or oval shape of the lumina, as compared to the large and irregularly shaped perivascular spaces, were of great assistance in locating the pinealocyte processes by light microscopy (Fig. 4 *a*, large arrows). Electron microscope examination showed that each lumen was outlined by the plasma membrane of one pinealocyte, but basement membrane material was not observed within these luminal spaces (Figs. 6 *b*, 8, 9, 12, 13). One pineal cell process was observed to extend into a perivascular space within the stroma (Fig. 7), without projecting into such a typical lumen.

The cells that lined the luminal spaces into which the bulbous processes extended were noted to have formed structures typical of the zonula adherens type of cell attachment about the necks of the bulbous processes (Figs. 6 *b*, *c*). These cell attachments completely encircled the cytoplasmic processes, and the diameters of the processes were smallest at the level of cell attachments. Mitochondria, rough-surfaced endoplasmic reticulum, and basal bodies of cilia within the bulbous processes were observed distal to this region of cell attachments (Figs. 7–9).

The pinealocytes that outlined the luminal spaces resembled, in some respects, those that formed the bulbous cytoplasmic processes projecting into the spaces. The cytoplasm was of equal density, with glycogen and ribosomes scattered throughout, and contained rounded and elongated mitochondria and microtubules that followed no regular pattern of orientation. These cells were also observed to develop elongated cell processes that closely resembled the bulbous cell processes they surrounded (Fig. 13). Occasionally, these pinealocytes that surrounded a lumen projected a cilium into another adjacent lumen (Fig. 12).

In the 12-day old animals, one pinealocyte process was observed in each lumen. In the 17-day old animals, however, these processes were observed in groups of four to eight and were arranged in a circular cluster. The elongated cell processes were aligned in a radial fashion with their ends tapering towards the center of the cluster and were no longer bulbous in appearance (Fig. 14). They were connected along a plane of cell junctions near the center of the cluster, where they extended into a constricted, irregularly shaped luminal space. These spaces often contained microvilli; few cilia or membranous structures, however, were observed in these spaces at 17 days of age. The morphologic features suggestive of photoreceptor cells in the 22-day old rats were less striking than those observed in the 17-day old animals. The bulbous cell processes and their distinct globular lumina found in the 4–12-day old rats were absent in the 22-day old group. Clusters of pinealocytes like those described in the 17-day old rats were observed gathering around small irregular lumina, and there was an absence of cilia and membranous structures within these lumina.

The pinealocytes of adult animals showed fur-

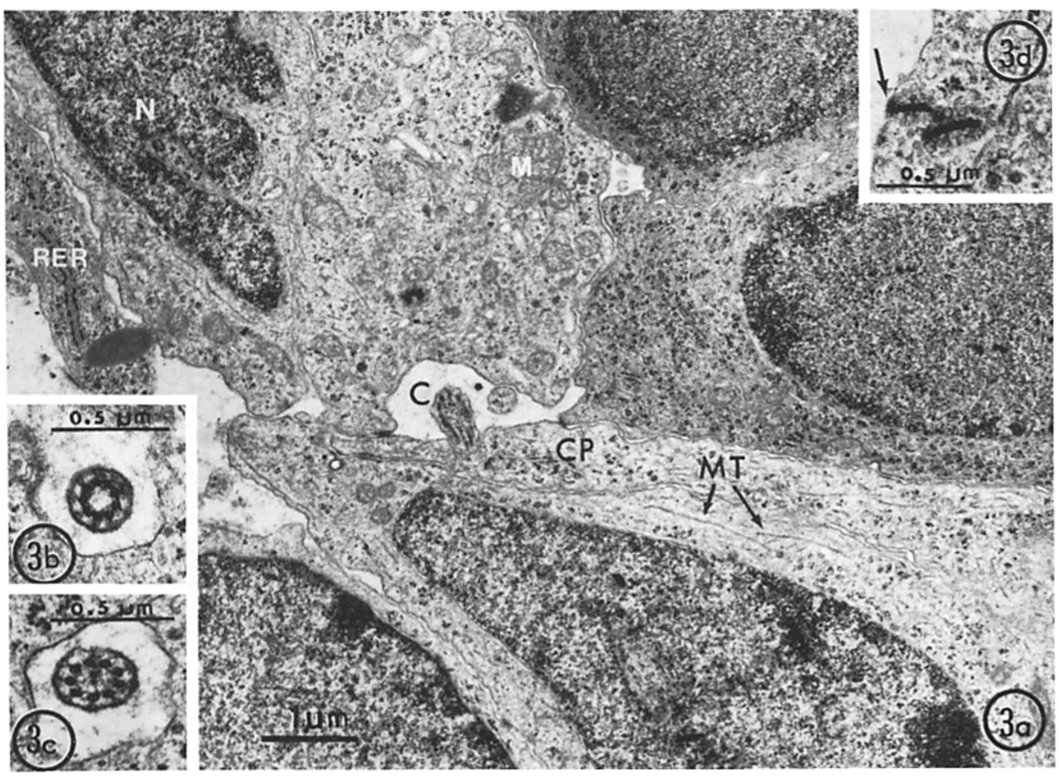


FIGURE 3 Pinealocytes of a 4-day old rat. Fig. 3 *a*, A cluster of parenchymal cells with typical cytologic features. One of the nuclei (*N*) shows invaginations of the nuclear membrane. In the cytoplasm, mitochondria (*M*), rough endoplasmic reticulum (*RER*), and microtubules (*MT*, arrows) are evident. A cilium (*C*) cut in tangential section projects from a cell process (*CP*) into an intercellular space. Fig. 3 *b*, A shaft of a cilium containing nine pairs of peripheral filaments but none in the center (9 + 0 pattern). Fig. 3 *c*, Shaft of another cilium containing nine pairs of peripheral and two of central filaments (9 + 2 pattern). Fig. 3 *d*, "Synaptic" ribbons located near the plasma membrane are surrounded by a halo of synaptic vesicles. One of these structures makes an arciform density (arrow) at its junction with the plasma membrane. *a*, × 13,000; *b*, × 31,000; *c*, × 33,000; *d*, × 25,000. AFIP neg no. 72-12003-3.

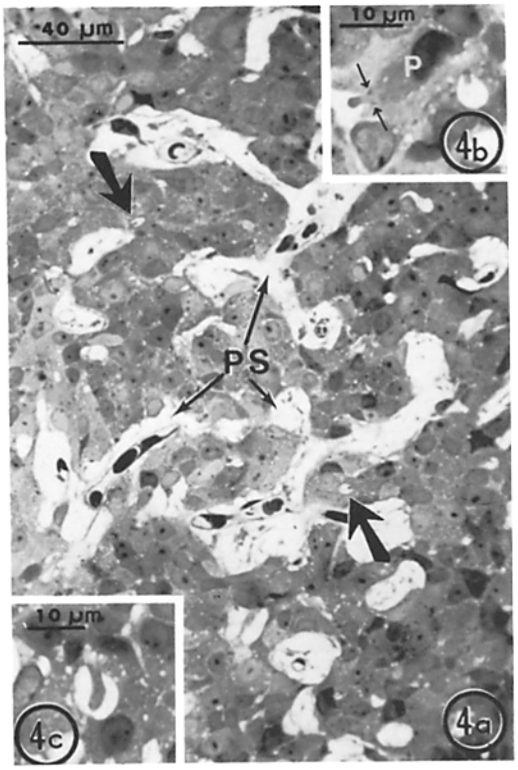


FIGURE 4 (Figs. 4–13 are taken from 12-day old animals.) Fig. 4 *a*, The pinealocytes form tenuous cords separated by connective tissue. Tiny oval luminal spaces (large arrows) containing protruding cell processes are located within the parenchymal tissue and are not continuous with the perivascular extracellular spaces (*PS*, small arrows) in the connective tissue septa. Fig. 4 *b*, A polarized pinealocyte (*P*) showing the nucleus located at one pole and, at the other pole, a bulbous cytoplasmic process extending into a luminal space. Cell junctions (arrows) surround the cell process. Fig. 4 *c*, A long cytoplasmic process of a pinealocyte within a lumen. *a*, × 350; *b*, × 820; *c*, × 750. AFIP neg. no. 72-12003-4.

ther alterations in form and structure. The nuclei of these cells were rounded, elongated, or polymorphous. Clusters of long cytoplasmic processes containing lipoidal bodies, elongated mitochondria, and microtubules oriented parallel to the long axis of the cell were observed in a rosette formation with a ring of cell attachments connecting their ends (Fig. 15). The lumina of these rosettes were very small and contained no bulbous cytoplasmic processes, cilia, or membranous structures. Occasional pinealocytes were observed to have dilated processes, but they did not project into a lumen but were surrounded by adjacent pinealocytes. Their cytoplasm did not contain basal bodies or centrioles and no cilia have been observed in the adult pinealocytes. Some pinealocytes contained "synaptic" ribbons that were located either near the nucleus or within a cytoplasmic process.

The morphologic study of the developing rat retina (Fig. 16) confirms the observations of Weidman and Kuwabara (28) and will not be described in detail in this paper; for comparative purposes, however, the morphologic characteristics of the developing photoreceptors in the 4-day old rat are described.

At 4 days of age, the retinal photoreceptors had developed rudimentary inner segments (Fig. 16). These consisted of short, bulbous cytoplasmic expansions containing round and elongated mitochondria, microtubules oriented parallel to the long axis of the cell, basal bodies, rough endoplas-

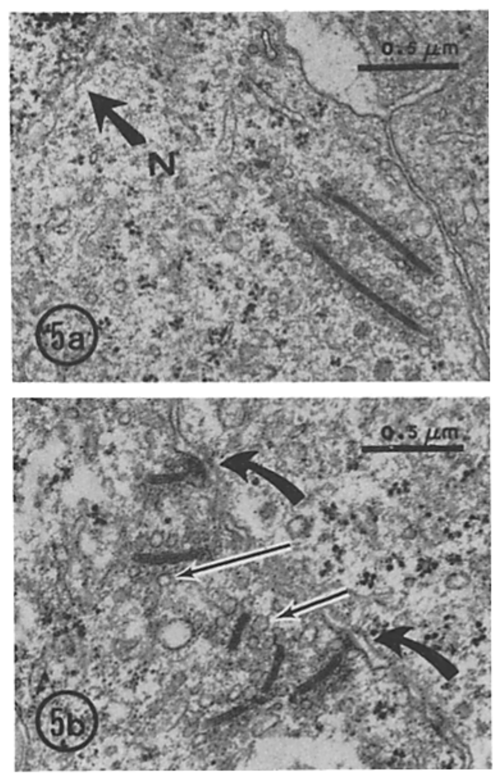


FIGURE 5 Fig. 5 *a*, "Synaptic" ribbons at the nuclear pole are situated proximal to the nucleus (*N*, arrow). Fig. 5 *b*, "Synaptic" ribbons with their surrounding "synaptic" vesicles (thin arrows) and arciform densities (thick arrows), which are formed at the plasma membrane. *a*, × 27,500; *b*, × 30,000. AFIP neg. no. 72-12003-5.

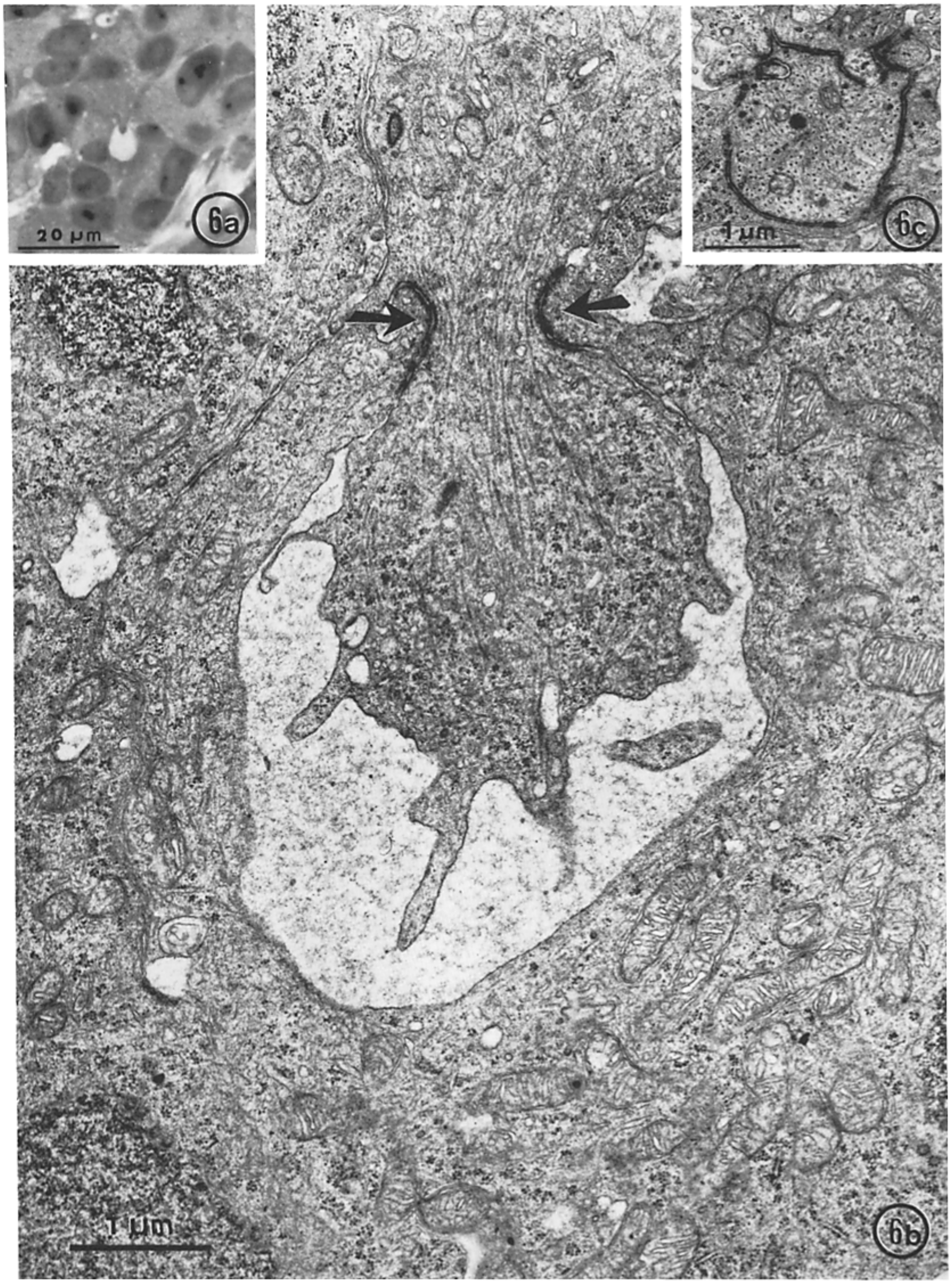


FIGURE 6 The bulbous cell processes of the polarized pinealocyte. Fig. 6 *a*, Typical appearance by light microscopy of a cell process extending into a luminal space. Fig. 6 *b*, An electron micrograph of a cell process similar to that illustrated in Fig. 6 *a*. The process is bulbous and contains numerous microtubules oriented parallel to its long axis. Ribosomes and vesicles are located near the apex, where villi extend from the process into a lumen occupied by an electron-dense floccular material. The cell that surrounds the lumen contains numerous mitochondria, microtubules, and ribosomes. Zonulae adherentes (arrows) are formed with the surrounding cell at the level of the smallest diameter of the cell process. Fig. 6 *c*, In cross section, the cell process is encircled by the cell attachments. Microtubules appear as small dots. *a*, × 750; *b*, × 21,000; *c*, × 14,000. AFIP neg. no. 72-12003-6.

mic reticulum, and free ribosomes. These inner segments extended parallel to one another from a plane of zonulae adherentes that connected them to each other and to adjacent Müller cells. At the ends of the inner segments, cilia of the 9 + 0 pattern protruded into the extracellular space between the photoreceptor cells and the retinal pigment epithelium. The tips of the cilia were dilated and projected numerous villus-like cytoplasmic processes that formed vesicles and lamellated membranes.

DISCUSSION

The morphologic features of the pineal organs of adult rats have been well described histologically and cytologically (27, 29–31) and are believed to be characteristic of a solid secretory organ, but the morphology of pinealocytes in the neonatal rat has not been thoroughly investigated. In the present study, a specialized differentiation, very different from that of the adult, was first observed at 4 days after birth and was no longer apparent at 17 days of age or later. During this transient period, the pinealocytes displayed many morphologic features resembling those of developing photoreceptors of the retina (28). The first evidence of this specialized and transient differentiation of the primitive pinealocytes was the polarization of the cells. At one pole an elongated cytoplasmic process developed. These cytoplasmic processes were most frequently observed in the 12-day old animals. Their general appearance has some similarity to that of those occasionally observed in the pineal body of the adult rat (27), which have been described as reminiscent of the inner segments of photoreceptors of lower vertebrates (6). The most characteristic features, however, were the regularity with which these processes were observed protruding into a lumen (Figs. 6, 8, 9) and the uniform appearance of the lumina themselves.

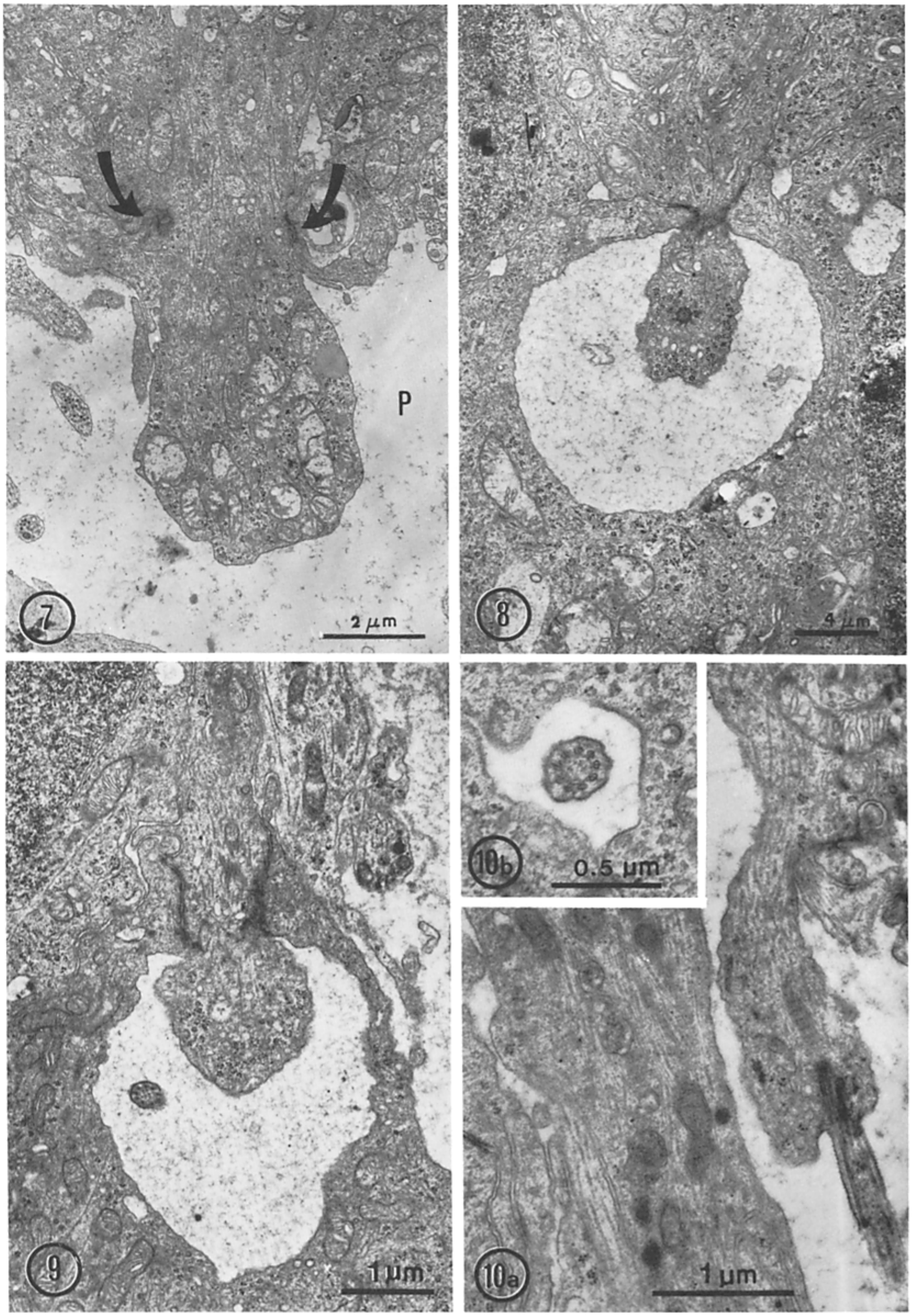
The cytoplasmic processes of the neonatal pinealocytes contain numerous microtubules, clusters of vesicles, and mitochondria. The retinal photoreceptors also contain microtubules and an aggregate of vesicles and mitochondria in the apical portion of the developing inner segments (28, 32). In comparison with the inner segments of the retinal photoreceptors, however, mitochondria in the pinealocytes are not as densely populated. On the other hand, the protrusion of these bulbous cytoplasmic processes containing rough endoplasmic reticulum, mitochondria, and basal bodies of cilia into a luminal space beyond the zone of cell junctions is remarkably similar to the structure of the ellipsoid of retinal photoreceptors. In contrast, the apical portions of the ependymal cells of the brain and the Müller cells of the retina (28, 33) that extend beyond the zone of their cell junctions are not bulbous but villus-like and devoid of the 9 + 0 pattern of cilia and mitochondria.

The cell junctions of the pinealocytes form a complete belt around the circumference of the cell processes and are exclusively of the zonula adherens type. These cell junctions are very much like those of the retinal photoreceptors, which are lined up in a series to form the so-called "external limiting membrane" of the retina (28). In contrast, the cell junctions of the ependyma and retinal pigment epithelium consist of both zonulae adherentes and zonulae occludentes (34). While the retinal photoreceptors develop in a very orderly plane that forms the "external limiting membrane," the pinealocytes develop singly in most instances and appear to be randomly oriented.

Structurally, the "synaptic ribbons" are identical with those observed in the photoreceptor cells of the retina, i.e. consisting of three dense layers, a halo of clear vesicles, and an arciform density (35). Furthermore, these ribbons appear in groups of two to six and therefore resemble those of the cone pedicles more than those of the rod spherules (35). In contrast to the synaptic ribbons of the retinal photoreceptors, which are located in the rod spherules or cone pedicles at the terminals of the axons, the "synaptic" ribbons in the developing pinealocytes are located within the cell body.

In confirmation of previous descriptions of "synaptic" ribbons in mammalian pinealocytes (29, 36), we did not observe these ribbons to be associated with any "postsynaptic" membrane thickenings or dense material within the "synaptic" cleft. For this reason, other terms such as "vesicle-crowned rodlets" have been used (37) in order to distinguish these structures from synaptic ribbons of typical photoreceptor cells. Recent work (24, 26), however, suggests that these structures are not merely phylogenetic relics but functional organelles, possibly involved in communication between adjacent pinealocytes. The term "synaptic" ribbon is therefore used in this paper.

Synaptic ribbons are specialized structures that are observed in a few specialized sensory cells, e.g. photoreceptors, bipolar cells of the retina, and the


P
7
2 μm
8
4 μm
9
1 μm
10b
0.5 μm
10a
1 μm

hair cells of the organ of Corti. The presence of these "synaptic" ribbons in the pineal body suggests that the pinealocytes are closely related to retinal photoreceptors and other specialized sensory cells of the body. This is in agreement with the concept that the mammalian pinealocyte belongs to a single cell line derived from the photoreceptive pineal cells of lower vertebrates (6). Thus, even though the mammalian pinealocyte is believed to have a secretory function (12), it should be regarded as a specialized neuron (38).

Distinctive ciliary structures were observed pro-

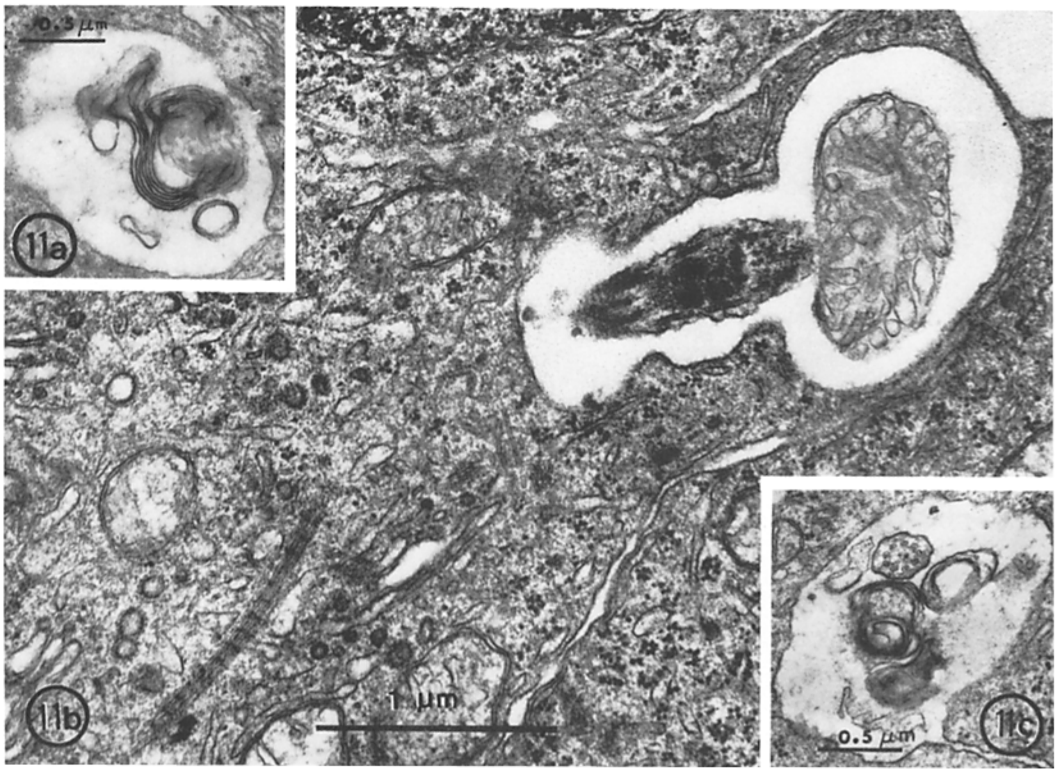


FIGURE 11 Membranous structures in the ovoid lumina that surround the bulbous ends of the pinealocytes. Fig. 11 *a*, Thick, lamellated membranes. Fig. 11 *b*, Vesiculated membranes lie adjacent to a cilium that is cut obliquely. A thin outer membrane confines the vesicles to a cluster that appears to extend from the cilium into a luminal space. Note the striated ciliary rootlet within the cytoplasm. Fig. 11 *c*, A cilium cut in cross section adjacent to lamellated membranous structures. *a*, × 23,000; *b*, × 32,000; *c*, × 22,000. AFIP neg. no. 72-12003-8.

FIGURE 7 A cytoplasmic process of a pinealocyte containing elongated mitochondria and microtubules oriented parallel to the long axis of the cell forms a bulbous end within a perivascular space (*P*). Note that the bulbous end extends beyond the cell junctions (arrows). × 7,800. AFIP neg. no. 72-12003-7.

FIGURE 8 A bulbous process containing a basal body of a cilium projects into a typical luminal space. × 3,200. AFIP neg. no. 72-12003-7.

FIGURE 9 A cilium cut transversely lies adjacent to a bulbous process. Zonula adherens-type cell junctions are prominent. × 14,000. AFIP neg. no. 72-12003-7.

FIGURE 10 A cilium extending from tip of cell process into a luminal space. Fig. 10 *a*, Longitudinal section of cilium. Fig. 10 *b*, In cross section, cilium displays nine pairs of peripheral filaments but lacks the central filament (9 + 0 pattern). *a*, × 22,000; *b*, × 33,000. AFIP neg. no. 72-12003-7.

FIGURE 12 A pinealocyte, which contains prominent microtubules, projects its apical process into a luminal space. A cluster of vesicles is located at the tip of the process; zonulae adherentes are formed with the cells that surround the process. This surrounding cell has developed a cilium of the 9 + 0 pattern, which projects into another lumen containing some lamellated membranous structures. × 26,000. AFIP neg. no. 72-12003-13.

truding from pinealocytes in the 4–12-day old animals but were rarely observed in older rats. In the 4-day old animals most of the cilia showed a 9 + 0 pattern in the shaft, but a few cilia of the 9 + 2 pattern are probably developed from cells that still retain cytologic characteristics of the lining cells of the neural tube.

The cilia that projected from the cell processes in the 12-day old group are exclusively of the 9 + 0 pattern. This pattern is typical of nonmotile sensory epithelia and is one of the characteristics of the retinal photoreceptors. Our findings are consistent with the previously reported existence of 9 + 0 ciliary derivatives of pinealocytes in fetal rats (19, 20) and the reported absence of such structures in adult rats (27). It appears that these structures represent a specialized but transitory differentiation of the pinealocytes.

Two types of membranous material were observed within the luminal spaces. The first and most frequently observed type consisted of whorls of disrupted membranes of uniform thickness, regularly stacked in a lamellated fashion. These disrupted membranes are reminiscent of the degenerative outer segment lamellae shed from the cilia of pineal photoreceptors in the frog during physiologic renewal (39).

The second type of membranous material consisted of vesicles confined to a cluster by an outer plasma membrane. These membrane-bounded vesicles appeared to extend from the tip of the cilium; direct continuity between the plasma membrane surrounding the vesicles and that of the cilium, however, is difficult to establish because of the random arrangement of the cilia and the resulting oblique sections.

These vesicular membranes are morphologically similar to the vesiculated outer segments that have been observed in developing retinal and pineal photoreceptor cells. During the morphogenesis of the retinal outer segments in kittens (40) and in rats (28), vesicles, which subsequently develop into photoreceptor lamellae, form at the tip of the cilium of the photoreceptor cell. Vesicles within the terminal expansion of a cilium have also been described in the pineal organs of certain birds and reptiles and are regarded as rudimentary outer segments (6). Thus, the vesiculated membranes observed in the pineals of the neonatal rats may represent a rudimentary form of outer segment development.

It should be noted, however, that a variety of stress factors may also produce vesiculation of normal outer segment lamellae of photoreceptor cells, e.g. exposure of rats to light (41), experimental detachment of the retina in the owl monkey (42), and even osmotic stress in tissue preparation (43–45). Vitamin A deficiency is also known to produce vesiculation of outer segment lamellae in the retinal rods of rats (46) as well as in the pineal eye of the lizard *Sceloporus occidentalis* (47). These considerations suggest another route to the formation of vesiculated membranes, i.e., that they may arise from the breakdown of previously formed lamellar membranes.

The specialized differentiation of the pinealocytes described above appears to mimic that of the developing retinal photoreceptor cells. The pinealocytes at 12 days of age, as well as the retinal photoreceptors at 4 days of age, develop "ellipsoid-like" (20) inner segments with well-developed zonulae adherentes and rudimentary outer segment structures consisting of cilia of the 9 + 0 pattern and vesicular and lamellated membranes. These observations suggest that the pinealocytes at this period are differentiating along the same

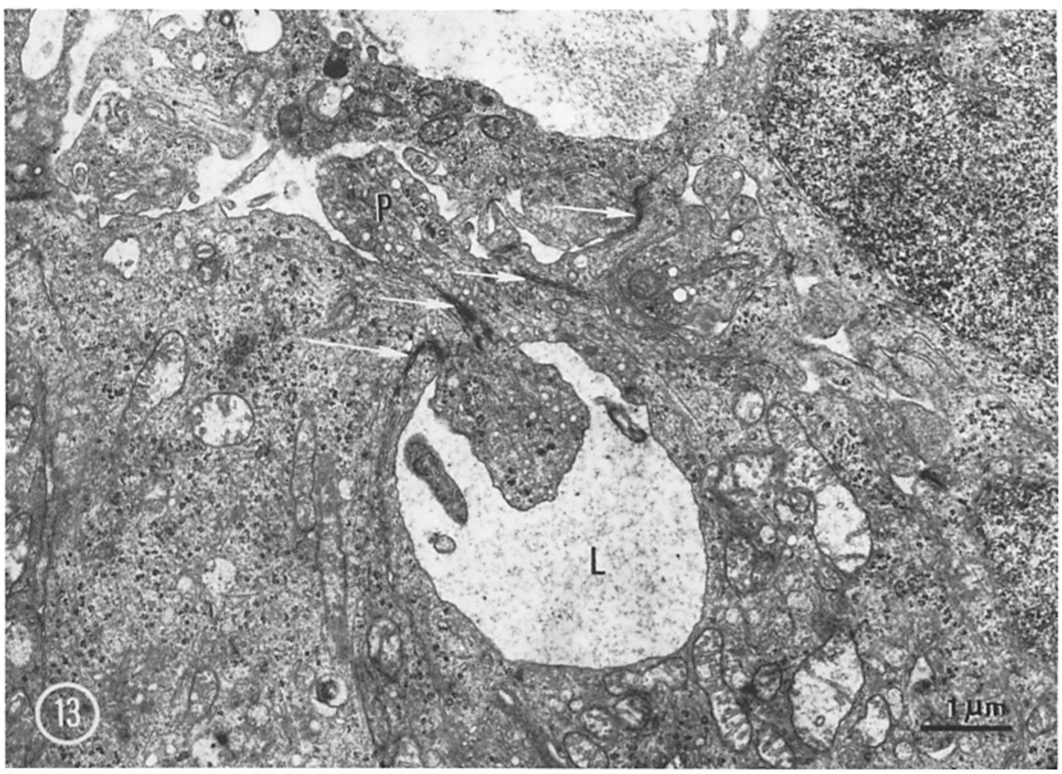


FIGURE 13 Two adjacent pinealocytes have apical bulbous processes that are projected in opposite directions. One process (*P*) is formed by the cell that also forms the lumen (*L*) into which the other process projects. Note the linear arrangement of cell junctions at the necks of the two processes (arrows). × 12,000. AFIP neg. no. 72-12003-10.

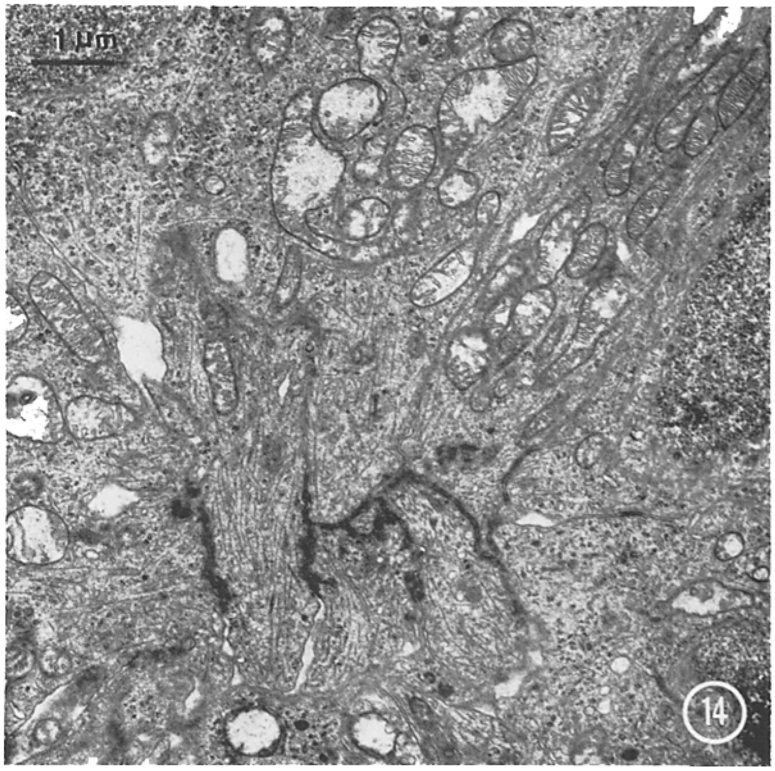


FIGURE 14 A cluster of pinealocytes in the 17-day old rat. Long processes containing numerous elongated mitochondria and microtubules converge in a circular array, forming a zone of cell junctions near their ends. × 11,000. AFIP neg. no. 72-12003-11.

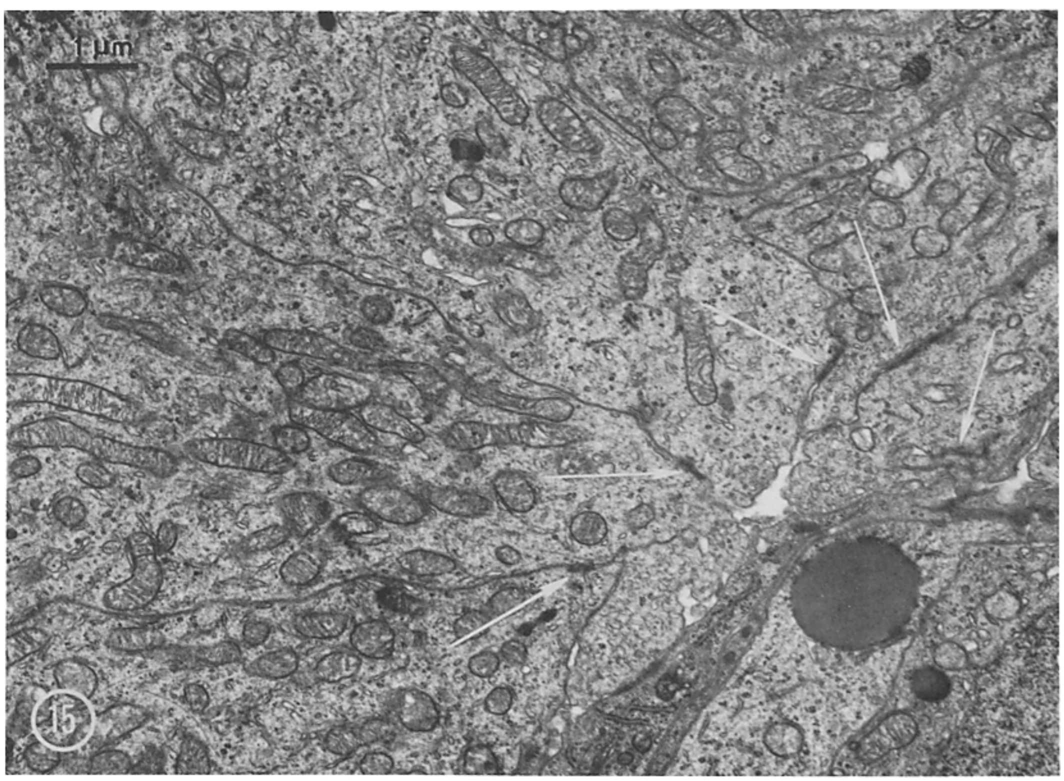


FIGURE 15 In the 96-day old adult rat, long processes of pinealocytes are arranged in a rosette-like formation. A ring of zonulae adherentes (arrows) is formed near the ends of the processes. Note the absence of mitochondria and microtubules in the small segments that extend beyond the ring of cell junctions. × 12,000. AFIP neg. no. 72-12003-12.

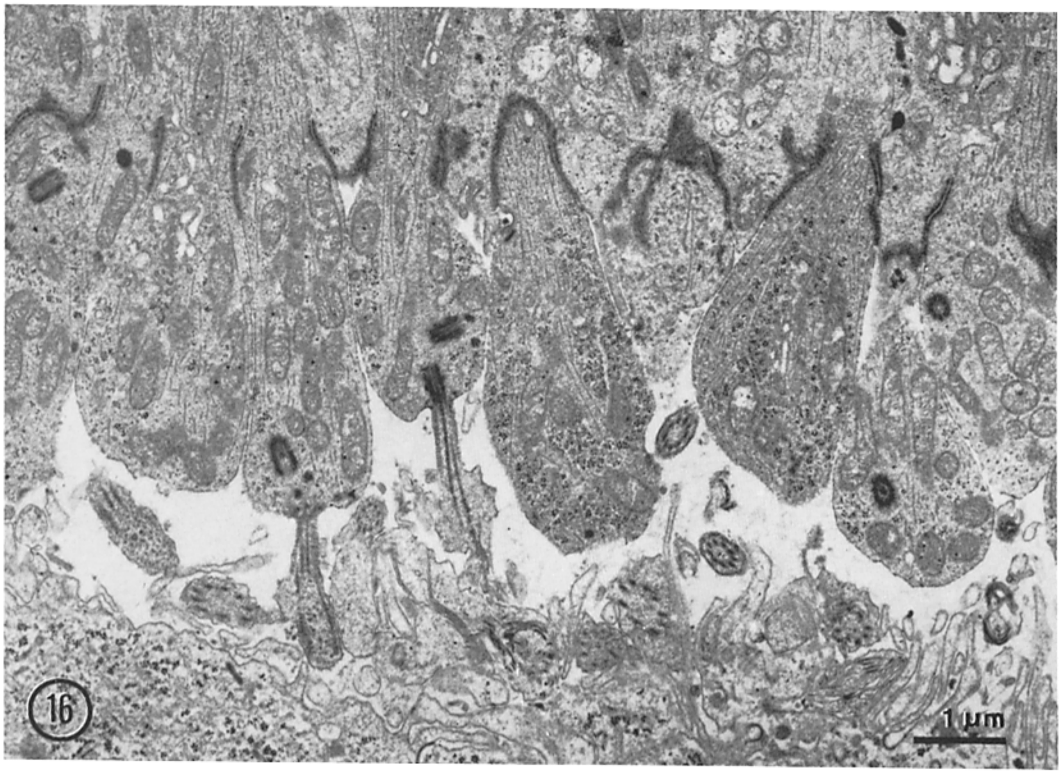


FIGURE 16 The developing photoreceptors in the retina of the 4-day old rat. Note the well-developed zonulae adherentes that form the outer limiting membrane. The immature inner segments consist of short bulbous processes containing basal bodies of cilia, a few elongated mitochondria, and microtubules oriented parallel to the long axes of the processes. The cilia are expanded at their tips and are just beginning to develop the lamellae of the outer segments. The typical 9 + 0 configuration is observed in cilia cut in cross section. × 12,000. AFIP neg. no. 72-12003-9.

direction as the retinal photoreceptors. This photoreceptor-like differentiation appears to be transient, since beyond 12–17 days of age the pinealocytes gradually lose their prominent inner segments, characteristic lumina, ciliary structures, and membranous material.

Clabough studied the development of the pineal in fetal rats and hamsters and described "surface modifications" of pinealocytes bordering on the lumen of the pineal anlage (20). These "modifications" consisted of "ellipsoid-like apical cytoplasmic bulges" with cilia of the 9 + 0 configuration, which were described as "reminiscent of developing photoreceptor cells." Unfortunately, Clabough did not continue her studies into the neonatal period and was therefore unable to observe the higher degree of differentiation, which we found to reach a peak at 12 days after birth.

This transient photoreceptor-like differentiation of the pinealocytes during the ontogenic development of the rat reflects the phylogenetic transformation of the pineal photoreceptor cells into pinealocytes, as previously suggested (48). The significance of this transient differentiation of the pinealocytes remains speculative; in view of the biochemical evidence of an extraocular photoreceptive site in the head (17, 21), however, the pinealocytes that show evidence of photoreceptor differentiation may be the photoreceptive site and may be capable, to some degree, of photoreceptive function. We must admit, however, that well-developed disks characteristic of outer segments are not observed, but it has been shown that immature photoreceptors with newly formed outer segments can elicit a response to light (49).

It is of interest that the retinal photoreceptors show a more advanced and orderly differentiation than the pinealocytes at all stages, though the eyelids are closed during this early neonatal period and are not opened until 14–17 days of age. During the neonatal period, the hair, skin, and subcutaneous tissues of the head are thin; the skull structures are not fused; and the pineal organ is more superficial than the retina. The pineal organ may be more accessible for stimulation by environmental light than the retinal photoreceptors.

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