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Embryonic Development of the Rat Pineal Gland

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ABSTRACT The embryonic development of the albino rat pineal gland has been studied from day 13 of development until birth. The first pineal anlage appears as a midline evagination of the diencephalic roof, which soon adopts a tubular morphology. At 17 days, the disappearance of the pineal recess begins, along with the transformation of the gland into a solid organ. The latter is mainly achieved by an infolding and thickening of the dorsal recess wall, from which derives most of the future pineal parenchyma. Blood vessels are mainly derived from the vessels found in the dorsal surface of the pineal gland.

The pineal gland of the albino rat has been extensively studied with a variety of biochemical, physiological and pharmacological techniques. One of the less examined aspects of the rat pineal gland is its embryonic development. In relatively recent times only two light microscope studies have been published on the embryonic development of the rat pineal gland (Kappers, '60; Clabough, '73). The principal objective of these studies, however, was not a systematic description of the embryonic development of the pineal gland. Kappers ('60) studied mainly the innervation of the gland. Clabough ('73) examined rudimentary photoreceptor structures in rat and hamster embryonic pinealocytes. Finally, Gardner ('53) and Machado et al. ('68), using the light microscope, studied the innervation of the rat pineal gland during development.

A detailed study of the rat pineal embryonic development may uncover basic features important for a better understanding of adult pineal morphology. In the present study we describe the light microscopy observations of the pineal gland development in the albino rat. The ultrastructural observations will be described in a separate study (Calvo and Boya, '80).

MATERIALS AND METHODS

Timed pregnant albino rats were sacrificed at 12-hour intervals from day 13 of embryonic development until day 21 post-coitum. The mother was anesthetized and the fetuses were removed from the uterine horns. Because of the obvious differences in size of the embryos in each litter, six fetuses were randomly sampled from at least two litters for the developmental stages studied.

After fixation in Bouin's fluid, the samples were embedded in paraffin, and serial sections 7 μm thick were obtained. The blocks were cut along frontal and sagittal planes to obtain longitudinal and transverse sections of the pineal gland. Hematoxylin and eosin, periodic acid-Schiff and silver impregnation techniques for reticular fibers were employed.

RESULTS

The anlage of the albino rat pineal gland observed with the light microscope first manifests itself in embryos of 13.5 days of embryonic development. The pineal appears as a short evagination located in the midline of the diencephalic roof anterior to the posterior commissure (Fig. 1). The pineal epithelium shows no detectable morphologic differences with the adjacent neuroepithelium.

At 15.5 days of embryonic development sagittal sections show the pineal as a tubular evagination having a clear posterior direction (Fig. 2). The pineal recess communicates widely with the third ventricle. Behind the pineal epithelium a well-differentiated posterior commissure and subcommissural organ are found (Fig. 2). The distal end of the pineal is attached to the dural venous sinuses.

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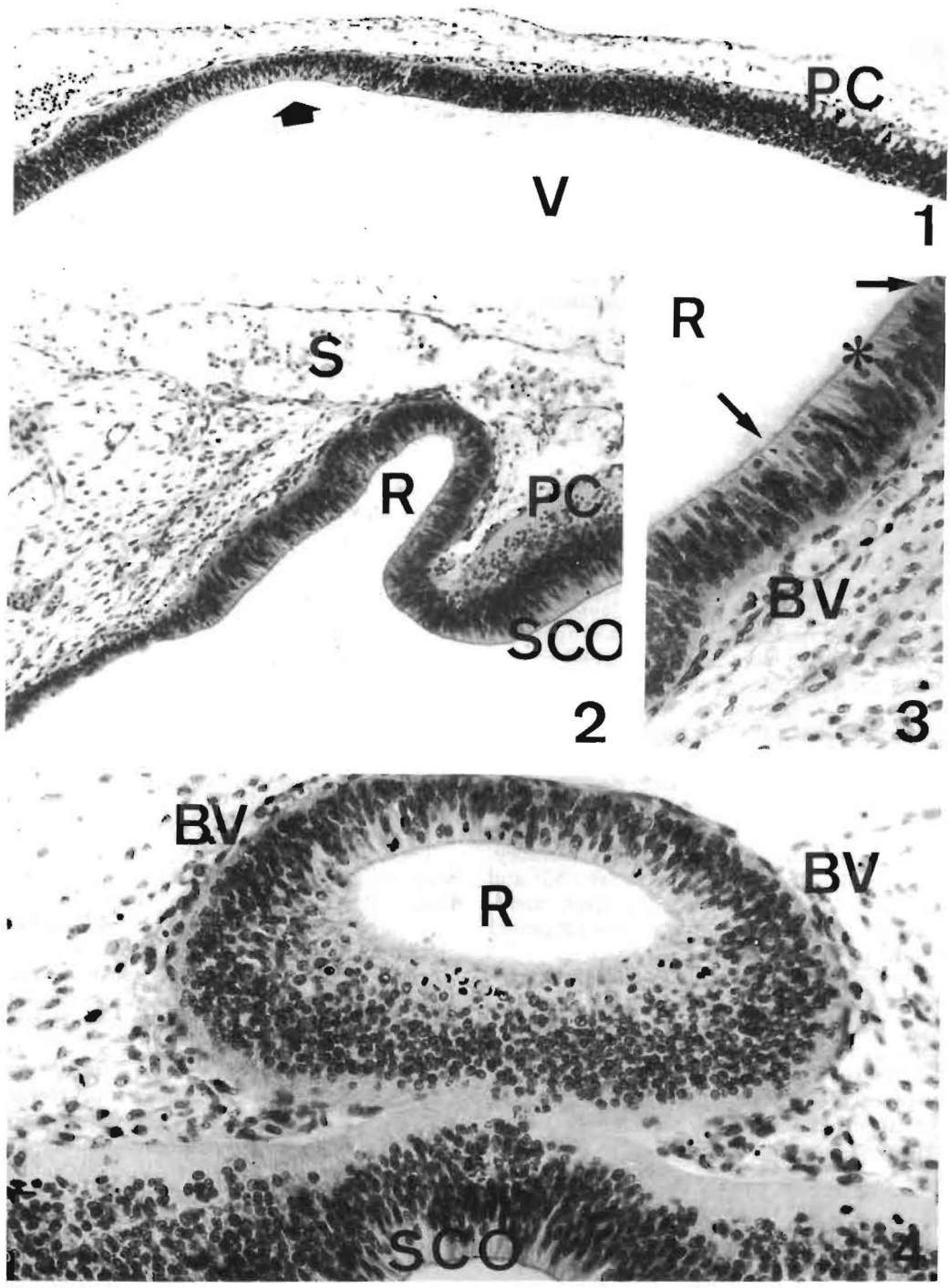
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At 15.5 days the pineal epithelium appears thicker, showing an increase in the number of nuclear layers. Near the recess lumen there are numerous mitoses, clear-cut terminal bars and apical pinealocyte projections (Fig. 3). From day 15.5 onward, degenerated cells may be found mainly in the basal region of the epithelium. They appear as eosinophilic globules with one or more intensely basophilic droplets inside. In transverse sections of the pineal gland the pineal recess presents an ovoid lumen that is flattened along its dorso-ventral axis (Fig. 4). Around the pineal evagination a regularly contoured basement membrane may be found.

At day 17 of development infolding of the pineal epithelium produces a groove in the roof of the recess. As a consequence, the lumen of the recess assumes an irregular shape, which is more prominent in the proximal two-thirds of the gland (Fig. 5). In the groove created, blood vessels may be found, some of which empty into the dural venous sinuses.

After day 18 of development, the pineal grows at a slower rate. The increase in size seems to be more prominent along the transverse axis. After 18 days of development transverse sections of the gland (Fig. 6) clearly show the obliteration of the pineal recess. Proximal sections (Fig. 6a) show an intensely folded pineal epithelium. In the midline, the infolding is more prominent, giving rise to a depression or groove in this region. This groove contains abundant small vessels in its depth. Sections (Fig. 6b) immediately anterior to the posterior commissure show that the groove is deeper here, and thus the lumen in the medial region of the pineal outline presents a smaller size. Lateral areas still show a wide lumen, although smaller than on day 17 of development.

At the level of the posterior commissure (Fig. 6c), the pineal is separated from the adjacent brain tissue by a meningeal sheet. The roof of the recess still shows its characteristic infolding. However, this dorsomedial region shows a marked thickening of the epithelium. Thus, both of these characteristics permit an approximation of the roof and the floor of the recess, which almost meet in the medial region. The lateral areas still show a wide lumen. In the superior regions of these areas, the walls tend to approximate and fuse, thus subdividing the lumen (Fig. 7). This process produces small cavities in which remnants of terminal bars of the pineal recess may still be found. Finally, an overall change in the contour of the pineal consists of a bending of the dorsolateral areas, which tend to direct themselves towards the medial groove (Figs. 7, 8).

At more distal levels (Fig. 6d, e, f), the previously described characteristics are more prominent. In the distal third of the pineal (Fig. 6f), the lateral areas of the pineal recess have already disappeared, and the lumen is reduced to a thin transversal fissure of poorly defined borders. This fissure narrows progressively, until it disappears in the distal end of the pineal. The parenchyma located above the recess, which derives from the recess roof, accounts for most of the pineal volume. The dorsolateral regions meet at the distal third of the gland, thereby leading to the disappearance of the medial groove.

One of the most characteristic changes in 18-day embryos is the appearance of mitotic figures in the interior of the pineal parenchyma distant from the lumen (Fig. 9). Apart from these mitoses, which we designate as "interstitial," evidence of cell divisions may still be found near the recess. The amount of intersti-

Abbreviations

BV, blood vessel
PC, posterior commissure
R, pineal recess
S, venous sinus
SCO, subcommissural organ
V, third ventricle

Fig. 1. Appearance of pineal evagination (arrow) in the diencephalic roof anterior to the posterior commissure anlage. 13.5-day embryo; sagittal section. × 80.

Fig. 2. Tubular shaped pineal evagination presenting a dorsal direction. 15.5-day embryo; sagittal section. × 160.

Fig. 3. Pineal epithelium with several nuclear layers. Existence of mitoses (arrows) and terminal bar (*) in the vicinity of the recess lumen. 15.5-day embryo; higher magnification of Figure 2. × 200

Fig. 4. Transverse section immediately anterior to the posterior commissure. Abundant periluminal mitoses. 15.5-day embryo. × 200.

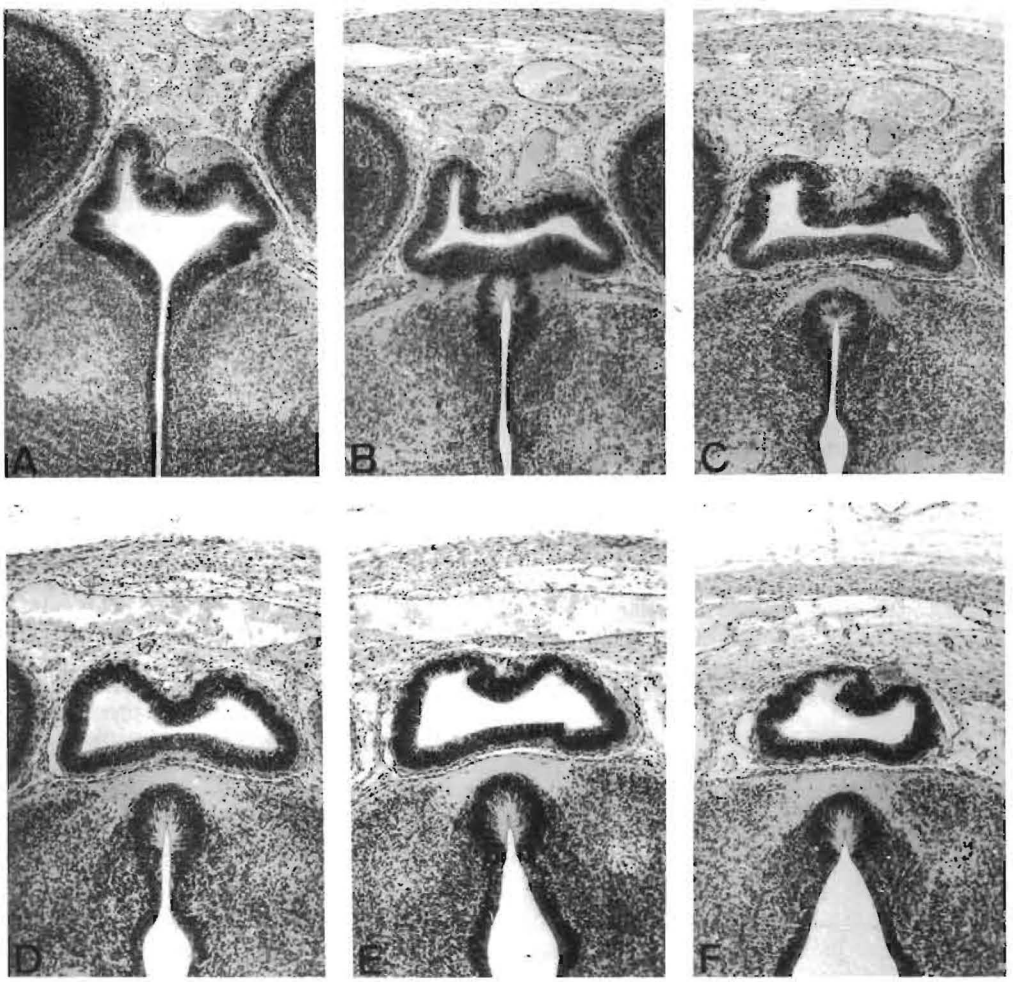


Fig. 5. Series of six transverse sections of the pineal gland taken at intervals of 100 microns beginning with the opening of the pineal recess to the third ventricle (section a). The point of reference taken for all the series of sections is the angle of union between the subcommissural organ and the pineal (section b). 17-day embryo. × 40.

tial mitoses varies along the pineal gland. They are scarce proximally, being found mainly in the dorsal region and near the basement membrane (Fig. 9). In the middle portion, they are located mainly in the medial thickening of the roof. In the distal third of the pineal they are more abundant than in proximal areas.

The thickening of the medial region presents other important characteristics. At this level, the luminal surface does not show the clear terminal bars found elsewhere around the recess (Fig. 8). The disappearance of bars is associated with the absence of periluminal mitoses. The area of the epithelial thickening shows pinealocyte nuclei forming circular arrays, but a central lumen in the interior is not found. The lateral regions show the same type of pinealocyte arrangement, but the pinealocyte band formations are more elongated, and a small lumen may sometimes be found (Fig. 8). As previously described, these cavities originate from the lateral regions of the pineal recess. In most of these spaces, terminal bars are not complete. In areas where terminal bars are not found, the cells seem to extend into the central lumen (Fig. 7).

Intrapineal vessels begin to appear in the

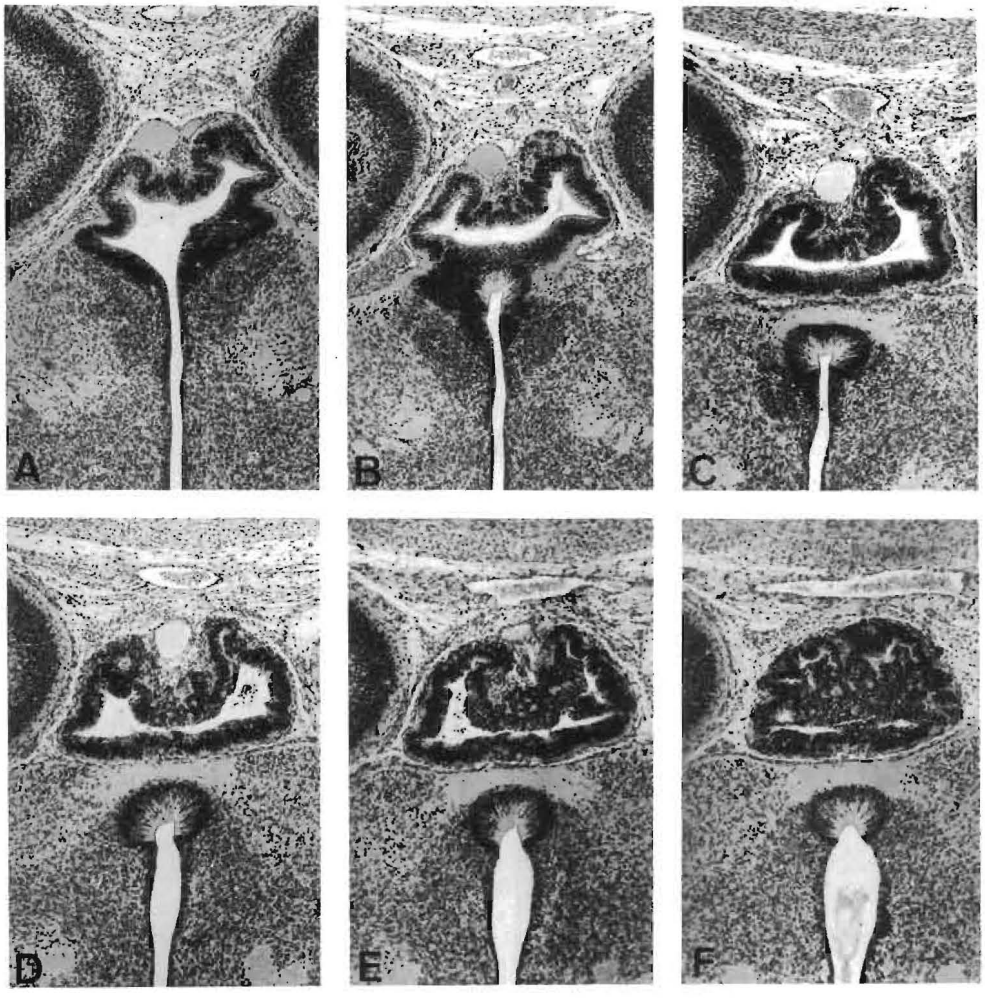


Fig. 6. Series of six transverse sections of the pineal gland. Same intervals and point of reference as in Figure 5. 18-day embryo. × 40.

pineal of 18-day embryos. Most of these vessels lie in the medial region above the recess, apparently deriving from the vessels in the depth of the groove (Figs. 8, 9). In the distal extreme of the pineal gland, vessels enter from the lateral regions outside the pineal. Silver impregnation for reticular fibers in embryos of 18 days show thin connective septa, coming from the periphery, penetrating into the gland. The more developed septa lie in the dorsal region and contain the above-mentioned capillaries.

All the changes described at 18 days of embryonic development continue to evolve rapidly in later stages. The obliteration of the pineal recess advances from distal to proximal regions (Figs. 10, 11). At 19 days the recess is found only in the proximal part of the pineal outline (Fig. 10). Immediately anterior to the posterior commissure, the recess appears in cross-sections as a transverse fissure located basally. The lateral regions of the recess are gone (Fig. 10b). Distally, the lumen progressively tapers down to a narrow fissure (Fig. 10c). At the same time, terminal bars are no longer visible near the lumen, beginning to disappear at the roof, later at the base, and

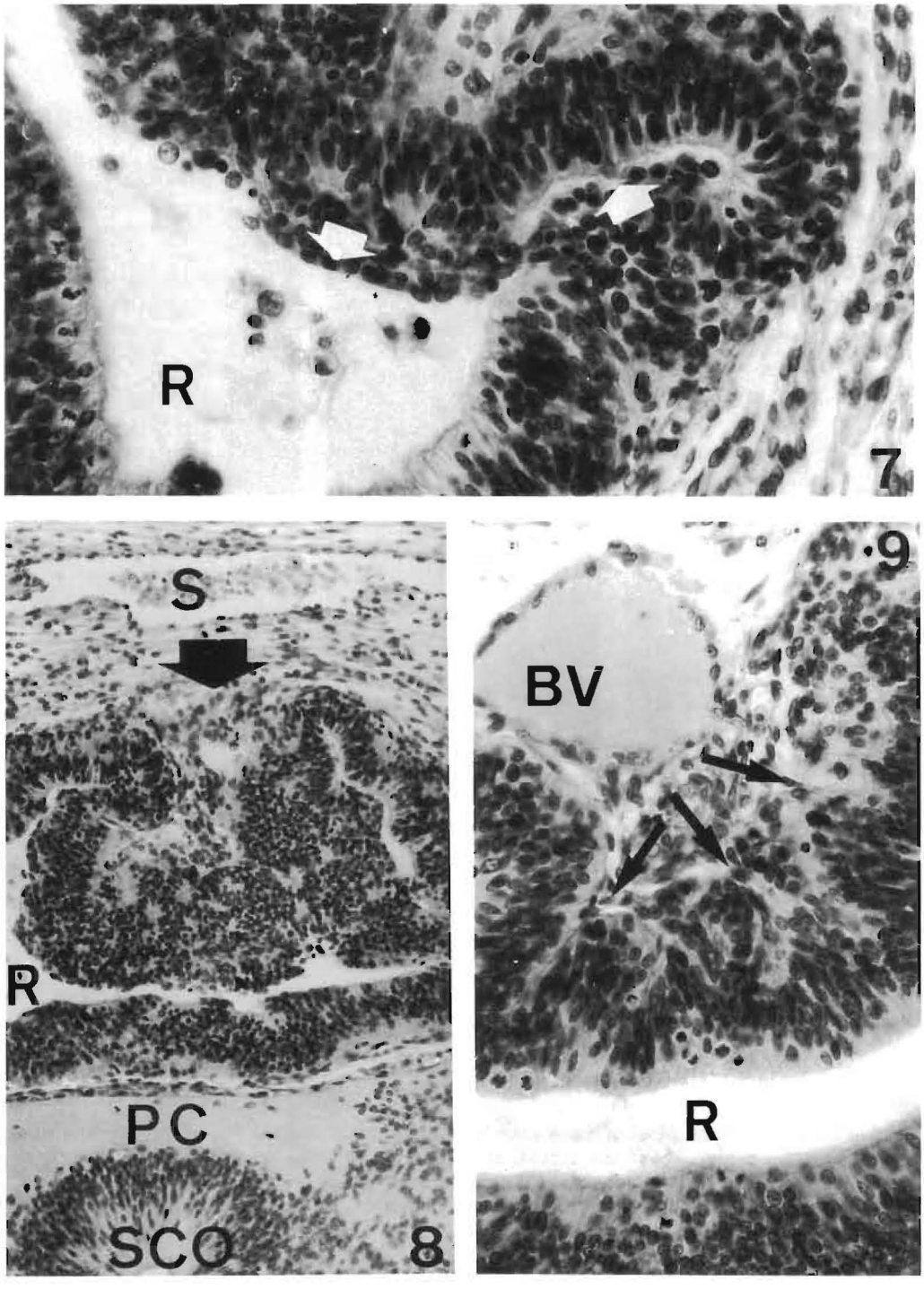


Fig. 7. Dorsolateral region of the pineal. Disappearance of the terminal bar in some areas of the wall (arrows). 18-day embryo; transverse section. × 320.

Fig. 8. Infolding and thickening of the dorsal wall of the pineal recess (arrow). Fragmentation of the lateral regions of the recess. 18-day embryo; transverse section. × 130.

Fig. 9. Dorsomedial region. Infolding of the pineal epithelium (arrows) presenting periluminal and interstitial mitoses. 18-day embryo; transverse section. × 260.

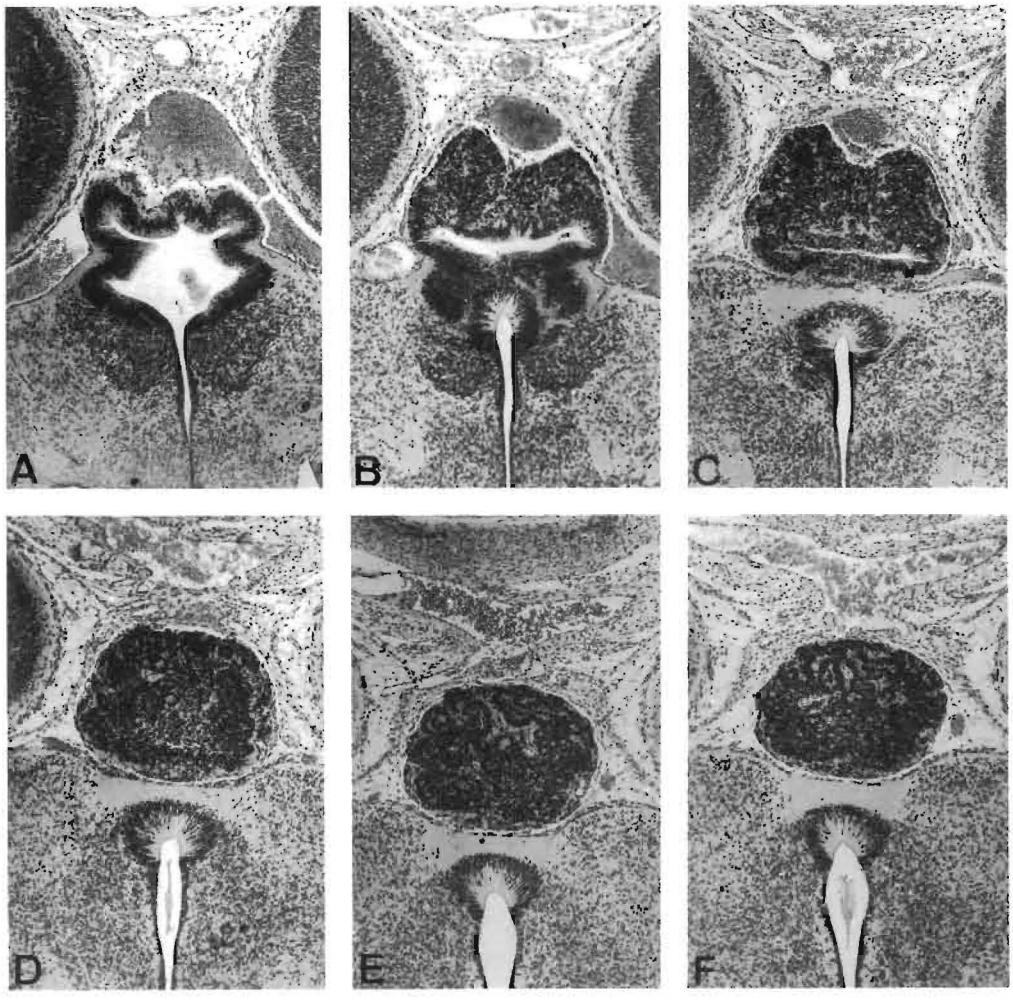


Fig. 10. Series of six transverse sections of the pineal gland. Same intervals and point of reference as in Figure 5. 19-day embryo. × 40.

finally at the lateral regions of the recess. At the moment of birth, the recess is found only in the proximal region of the pineal.

From 19 days onward, most of the gland presents a compact appearance (Figs. 10, 11, 12). Basally, cellular density is great, and the pinealocytes are arranged in a regular manner. The amount of mitoses and blood vessels here is less than that found dorsally. In the dorsal region the pinealocytes frequently form circular arrays, apparently without a central lumen (Fig. 12). Remains of terminal bars may still be found in some of these formations. With time, these remains progressively disappear, and the pinealocyte arrays decrease, giving the parenchyma a more compact appearance. However, in the last stages of embryonic development differences may still be found in the arrangement of the pinealocytes between the basal and dorsal regions. The lesser nuclear density found in the dorsal region is due to the greater amount of pinealocyte cytoplasm in this region (Fig. 12). The mitoses and capillaries seem to increase progressively until birth.

Silver impregnation techniques show a progressive penetration of connective tissue septa, which is more intense dorsally (Fig. 13).

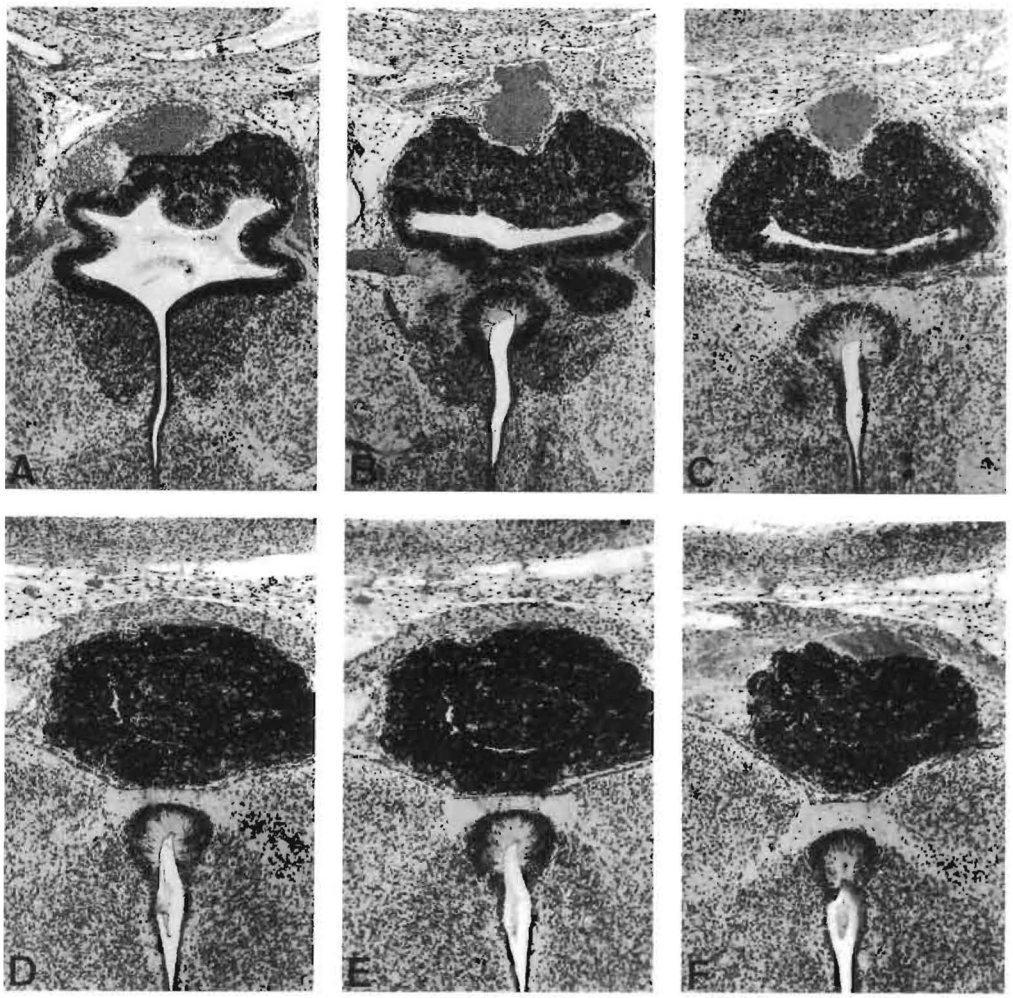


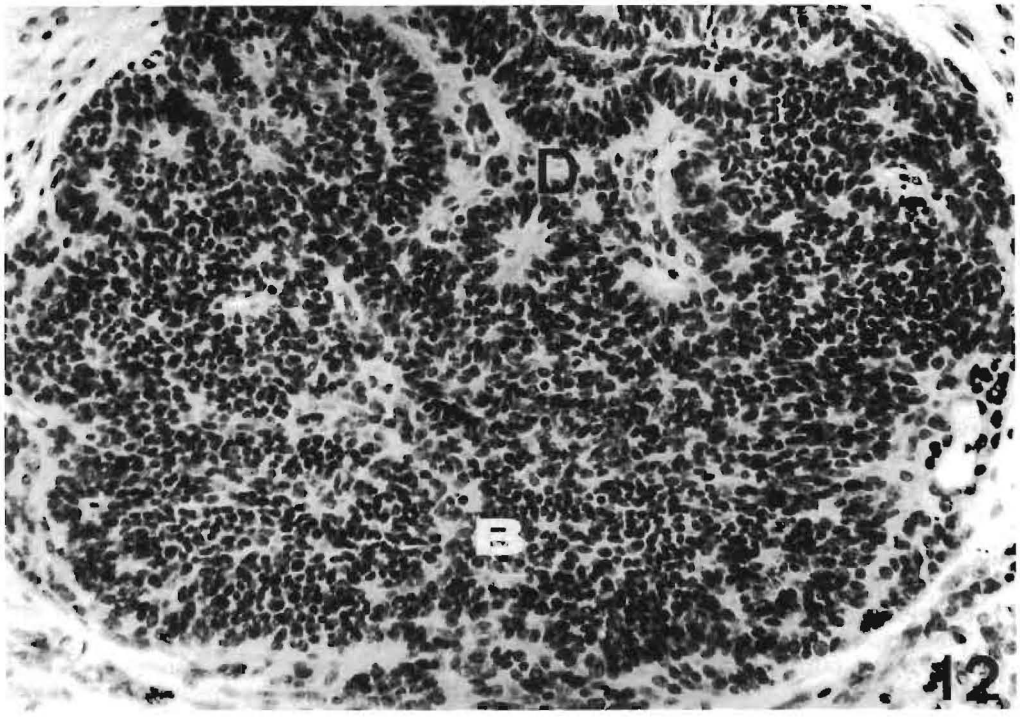
Fig. 11. Series of six transverse sections of the pineal gland. Same intervals and point of reference as in Figure 5. 20-day embryo. × 40.

DISCUSSION

Our results show that the pineal anlage may be recognized in rat embryos by 13.5 days of development. This primordium can be identified only on a topographic basis, since its epithelium may not be differentiated microscopically from the surrounding neuroepithelium. The simultaneous appearance of the pineal evagination with the initial differentiation of the posterior commissure permits a definite identification of the pineal evagination. Kappers ('60) describes a shallow pineal evagination in rat embryos of 14–14.5 days. Clabough ('73) confirms this date of appearance, but begins description of the gland in embryos of 16 days.

From its initial appearance at 13.5 days to 17 days, the pineal anlage grows into a tubular evagination. At the same time its wall thickens progressively. Intense periluminal mitotic activity is responsible for growth during this elongation phase. From a morphological point of view, the development of the rat pineal gland in these initial stages is comparable to our findings in the chick (Calvo and Boya, '78).

After 17 days of development, important changes take place in the configuration of the pineal anlage, which will become a solid organ.



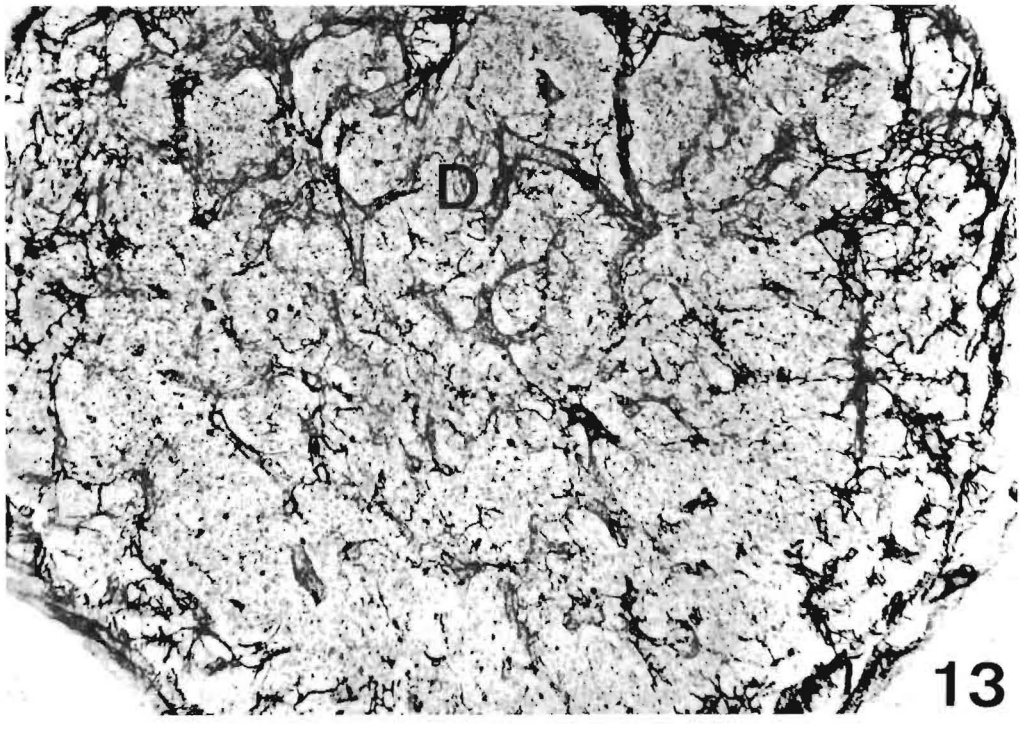


Fig. 12. Transverse section at the level of the posterior commissure. The basal area (B) shows a homogeneous arrangement of the pinealocytes, and the dorsal area (D) presents remains of the primitive recess. 19-day embryo. × 200.

Fig. 13. Vascular-connective stroma is more developed in the dorsal region (D). Birth; silver impregnation. × 200.

Our dating of this rearrangement of pineal morphology agrees with that of Kappers ('60). The pineal assumes its characteristic compact appearance as a result of infolding and thickening of the epithelium. These two processes are not necessarily consecutive, being concurrent at certain developmental stages, beginning in the distal part of the gland and advancing proximally. The obliteration of the recess takes place by fusion of its walls, preceded by the disappearance of terminal bars. The closing of the recess forms subsidiary cavities characterized by their orientation in the direction of the old recess and the presence of clear terminal bars, which are frequently found in only one of the two contact surfaces. In sagittal sections, these cavities appear isolated, and were interpreted by Kappers ('60) as the formation of follicles in the dorsal wall of the pineal. However, our results show that these cavities originate from the obliteration of the pineal recess; they are not new cavities formed in the depth of the epithelium. The mechanism of parenchymal growth by formation of rosettes has been described by us in the embryonic development of the chick pineal (Calvo and Boya, '78). Although we do not discard the possibility of a similar mechanism in the rat, all the cavities we see in the rodent appear to be derived from the pineal recess.

Two different explanations can be offered for the infolding of the pineal epithelium. The first is a mechanical limitation of pineal growth. After 17 days of development, the pineal occupies a narrow space limited by neighboring structures. Its growth in such a space would produce the observed infolding of its epithelium. However, an exclusively mechanical infolding would affect the entire gland and produce infoldings along the pineal surface. The second explanation for infolding would be differential mitotic activity, which is one of the most invoked morphogenetic mechanisms. According to this mechanism, areas of lesser proliferation would tend to infold towards the lumen. Further support for this interpretation comes from the disappearance of terminal bars and associated periluminal mitotic figures at the level of infolding. However, we do not know whether this is a cause or a consequence of infolding.

Until 18 days of development, the increase in volume of the gland is due to periluminal mitosis. Thereafter, interstitial mitoses take place. Moreover, the increase in pineal parenchyma is not accompanied by increase in pineal volume. The thickening of the epithelium takes place at the expense of the already reduced lumen, resulting in the rapid closing of the pineal recess. After 18.5–19 days, the recess is reduced to a narrow proximal fissure. There is also an increase in the fragmentation, closing and disappearance of the cavities formed by the fusion of the recess walls. However, even in advanced phases of this process, remains of terminal bars and pinealocyte array formations may still be found.

Finally, the majority of changes in the pineal after 17 days almost exclusively affect the dorsal portion of the gland, corresponding to the roof of the pineal recess. Thus, most of the rat pineal gland is derived from the roof. In this sense, rat pineal development is similar to that of the chick (Calvo and Boya, '78).

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