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Embryonic development of the pineal gland of the chicken *(Gallus gallus)*

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Key words. Pineal gland · Chicken · Embryonic development

Abstract. A study of the embryonic development of the pineal gland of the chicken was performed with the optical microscope. The time of apparition of the first outline, as a derivative of the roof of the third ventricle, was fixed at 3 days. The major portion of the pineal parenchyma is derived from the frontal wall of the outline. All the vesicles and follicles were formed by solid mammilliform projections which subsequently presented a central lumen. In no case was a communication of the follicular cavities with the pineal recess observed. Three categories of vesicles or follicles can be distinguished according to their origin.

Introduction

A great interest in the pineal gland has developed during the recent years. The morphological investigations have been centered fundamentally on the pineal gland of mammals and, to a lesser degree, on those of lower animals (fish, amphibians, reptiles). The pineal gland of the bird occupies a key position in the phylogenetic evolution of this organ. It is located between the sensorial pineal glands which are endowed with photoreceptors, as in the inferior vertebrates, and the secretorial pineal glands of the mammals which lack sensorial cells but have an endocrine function [*Ariëns Kappers and Schade,* 1965; *Wurtman et al.,* 1968; *Wolstenholme and Knight,* 1971; *Relkin,* 1976]. Because of this, very little attention has been dedicated to the pineal morphology of the birds. It is, therefore, evident that it is necessary to find a morphological model, at the level of the optical and electron microscope, of the pineal gland of the birds, which includes besides the embryonic developement the post-incubation evolutionary process. From this information, we could evaluate the pineal responses in diverse experimental situations. Of all the birds, the chicken is the most easily available and the most commonly used for embryological, biochemical, physiological, etc. studies.

The embryonic development of the pineal gland of the chicken has been little studied with the optical microscope. Discounting some fundamentally anatomical communications at the beginning of this century [*Hill,* 1900; *Cameron,* 1903; *Studnička,* 1905; *Funkquist,* 1912], there is only one relatively recent work [*Spiroff,* 1958] in which some of the morphological aspects of the embryonic development of the pineal gland of the chicken are described.

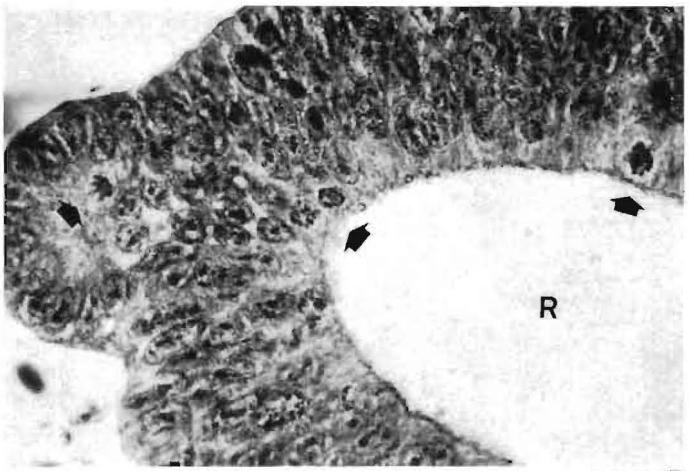

Fig. 3. Embryo of 5 days. Pineal outline in which frequent mitoses can be seen (arrows) in the vicinity of the lumen of the recess (R). Note the existence of a mammilliform projection with the typical rosette disposition of its cells, without a central cavity. HE.

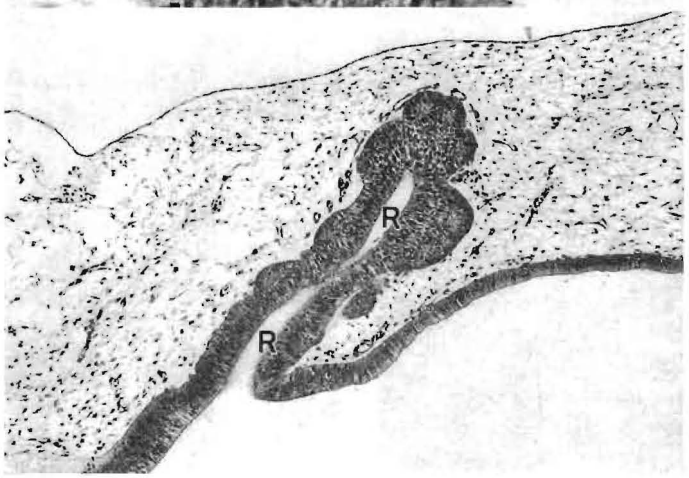

Fig. 4. Embryo of 5.5 days. Decrease in the lumen of the pineal recess (R). Larger quantity and development of the mammilliform projections. HE.

with the ventricular lumen, is observed. At high-power magnification, the cells of the outline demonstrate a basal nucleus and an ample apical cytoplasm. There are abundant periluminal mitoses.

Stage 25 (4.5–5 Days)

The pineal evagination presents a round form with a diameter of 150–160 μm. The central lumen is ample as is the orifice of communication with the third ventricle. The epithelium of the outline is thicker than that of the roof of the neighboring diencephalon. We observe a greater thickness in the anterior surface of this outline.

Stage 26 (5 Days)

We start to visualize the posterior or caudal commissure behind the pineal outline. The most characteristic feature of this stage consists of the appearance of the cellular mammilliform projections which are oriented in the opposite direction from the lumen of the recess (fig. 2). This gives an embossed aspect

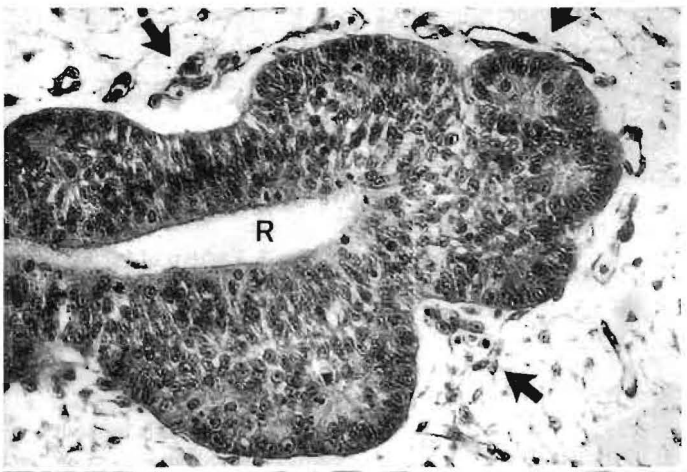

Fig. 5. Embryo of 5.5 days. A portion of the pineal outline in which the distal portion of the recess (R) and the better development of the cellular mammilliform projections, still without a central lumen, are seen. Around the outline, a capillary net begins to differentiate (arrows). HE.

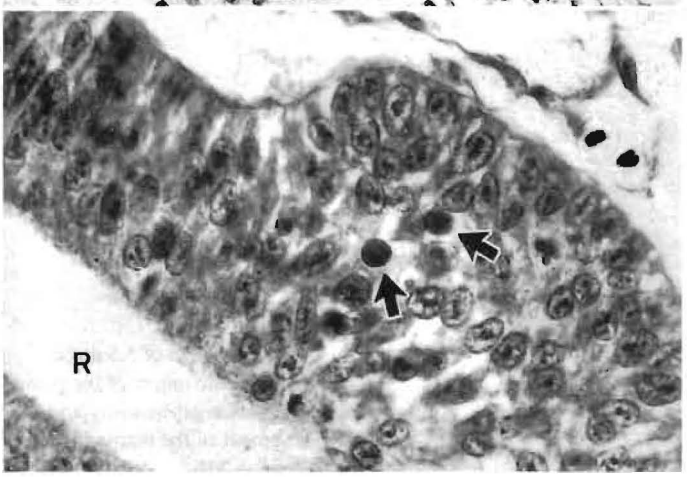

Fig. 6. Embryo of 5.5 days. Dense acidophilic bodies (arrows) in the pineal epithelium. R = Recess. HE.

to the contour of the gland. At high-power magnification (fig. 3), these mammilliform projections show a rosette cellular disposition; we still do not see a central lumen. Nevertheless, mitosis is encountered in the center, in the vicinity of the pineal recess.

Stage 28 (5.5–6 Days)

The differentiation of the choroid epithelium starts in the anterior portion of the pineal outline. This outline presents an elongated form, 430 μm in length and 140–180 μm in width. There is a clear inclination of the axis of the pineal gland in the frontal direction (fig. 4). Surrounding the pineal outline we found a poorly defined vascular net. The wall of the pineal recess demonstrates the cellular mammilliform projections which are more abundant and larger than in the last phase (fig. 5). They are localized especially on the anterior extremity and the anterior, inferior (or frontal) wall of the outline. There still are no lumens in the center of the cellular mammilliform projections.

At high-power magnification, we found a few (fig. 6) large, round, intensely acidophilic

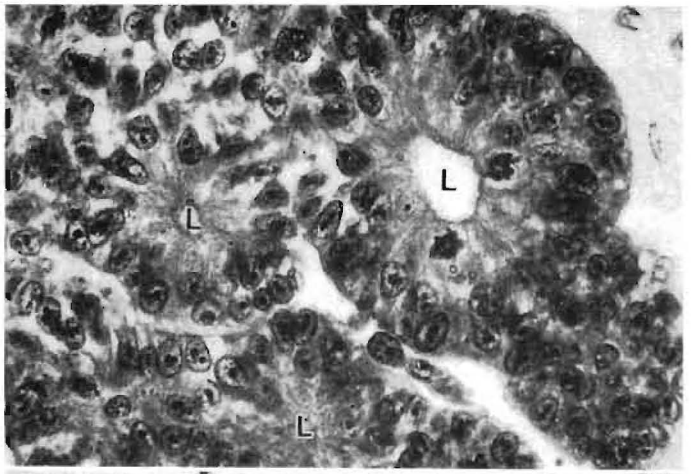

Fig. 7. Embryo of 6.5 days. Cellular mammilliform projections of the frontal surface of the pineal outline. Note the appearance of the central lumen (L). HE.

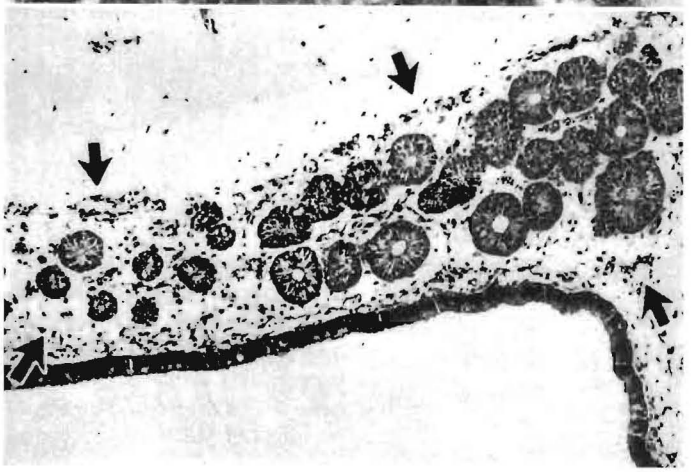

Fig. 8. Embryo of 7.5 days. The pineal outline limited by a capsule composed principally of blood capillaries (arrows). Greater density of the mesenchyme that will form the future pineal stroma. Numerous vesicles with central lumens. HE.

corpuscles of granular structure, which occasionally demonstrated one to three spherical, basophilic grains in their interior.

Stage 29 (6–6.5 Days)

The growth of the pineal outline continues and now it appears to be surrounded by a thick vascular net which is clearly delimited. Lumens start to appear in the cellular mammilliform projections (fig. 7), especially in the extremity and frontal surface of the outline, by which process these mammilliform projections are converted into vesicles. The pinealocytes have an ample apical cytoplasm. The lumens of the pineal recess and the vesicles show a very clear contour. There are abundant acidophilic corpuscles with basophilic granules, similar to those described above.

Stage 32 (7.5 Days)

The pineal outline is now perfectly defined. This is due to the existence of a capsule integrated with a dense vascular net (fig. 8) between which are abundant, flat, mesenchymal cells. Also, the pineal stroma is denser and more cellular than the neighboring

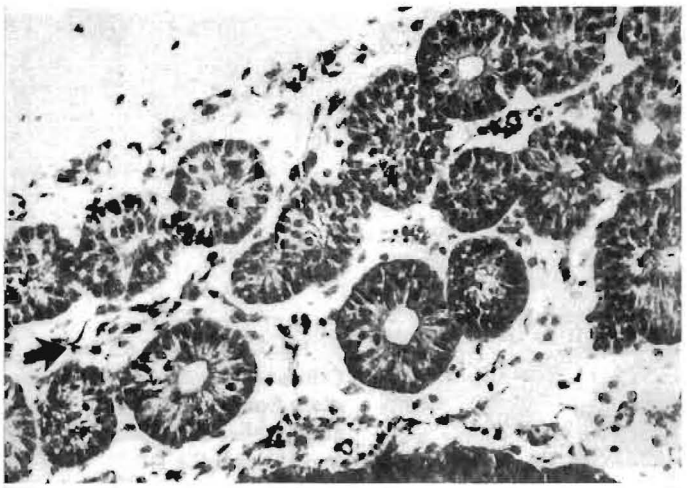

Fig. 9. Embryo of 7.5 days. Vesicles of the frontal surface of the pineal outline in which two layers of nuclei can be seen. In the intervesicular stroma, blood capillaries (arrow) are seen. HE.

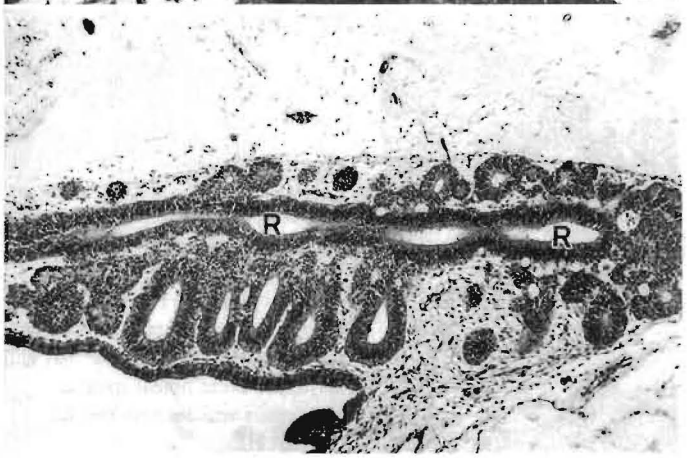

Fig. 10. Embryo of 11 days. Sagittal section of the pineal gland in which the recess (R) appears as a tubular formation with a small lumen. Great numbers of vesicles, especially on the frontal surface of the recess, at which level appear the vesicles of larger caliber. HE.

mesenchyme. We saw numerous clear vesicles from the central lumen on the frontal surface of the outline (fig. 8). In the larger vesicles, the nuclei of the pinealocytes are arranged in two layers (fig. 9), although apparently all the cells are in contact with the lumen. There are numerous periluminal mitoses next to the vesicular lumen. In this stage, the capillaries are encountered in the intervesicular stroma (fig. 9).

Stage 35 (8.5–9 Days)

The number of vesicles continues to increase due to the apparition of central lumens in the cellular mammilliform projections. At the same time, the median size of the vesicles has also increased.

Stages 37 and 38 (11 and 12 Days)

The highest index of growth of the pineal outline is encountered in these two stages (fig. 10, 21). The pineal gland reached a length of 1,200–1,300 μm and a width of 400–500 μm. The recess appears as a long, narrow, tubular cavity with a diameter of 50–60 μm. This recess continues with the third ventricle by means of a narrow communication which is

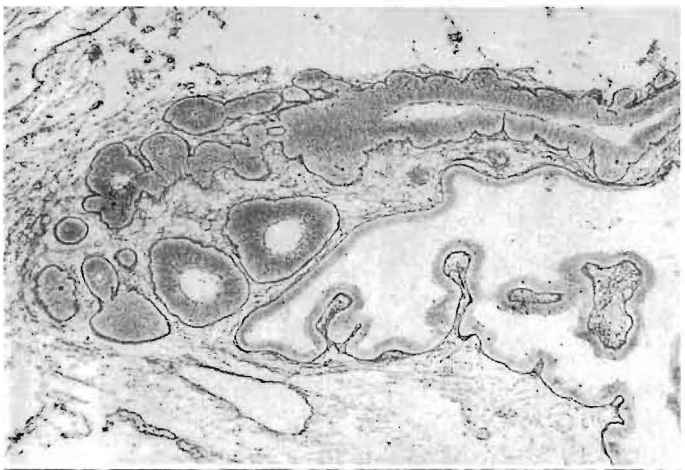

Fig. 11. Embryo of 11 days. The basal lamina around the recess and vesicles. Stroma with small quantity of fibers. Technique of Gomori.

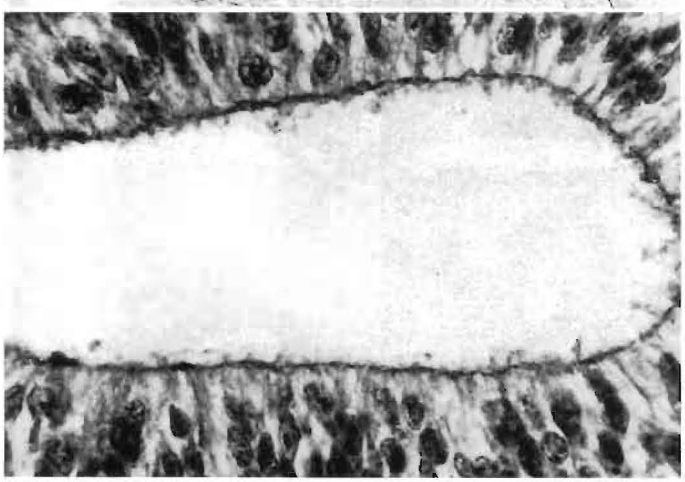

Fig. 12. Embryo of 11 days. Pineal recess. Apical projections of the pinealocytes toward the lumen of the recess. HE.

between the anterior or habenular and posterior or caudal commissures.

The pineal gland has a great number of vesicles which are more abundant on the frontal surface and on the extremity of the outline. Therefore, the pineal recess is clearly displaced toward the posterior-superior or dorsal surface of the gland. The larger vesicles are located on the frontal surface, arranged perpendicularly to the pineal recess. The dorsal surface only has a few small vesicles (fig. 10).

The PAS technique and the Gomori technique for reticular fibers show the existence of an evident basal lamina around the vesicles and the pineal recess (fig. 11). The pineal stroma appears very lax and poor in reticular fibrils. A marked increase in the number of intrapineal vessels was also noted.

The walls of the vesicles, especially of those of larger caliber, located on the frontal surface, are thicker than the walls of the pineal recess. The vesicular lumen and the lumen of the recess both present a few very pure limits (fig. 12). The pinealocytes possess filiform apical projections in these lumens.

In the stages 37 and 38, we started to dis-

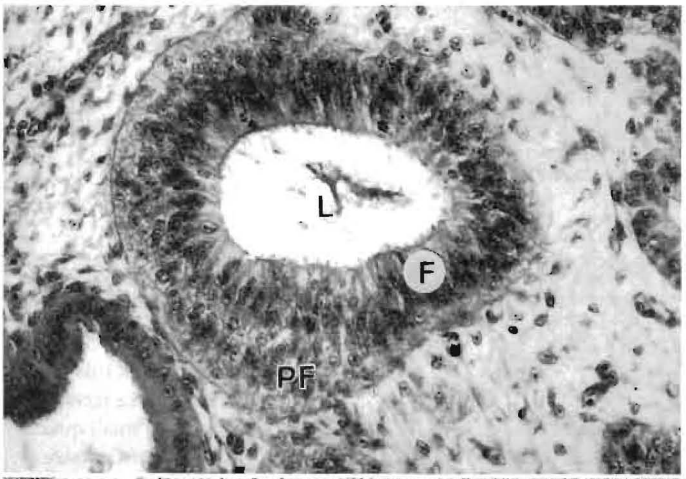

Fig. 13. Embryo of 11 days. Vesicles of the frontal surface of the recess in which a follicular zone (F) surrounding the central lumen (L), and the beginning of the formation of the parafollicular (PF) zone, next to the basal membrane and without relation to the central cavity, can be seen. HE.

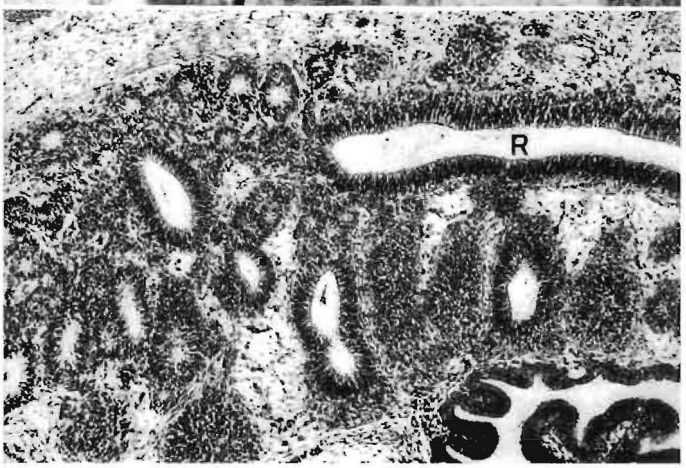

Fig. 14. Embryo of 13 days. Pineal gland with a compact aspect due to the increase in follicles and decrease of the stroma. Pineal recess (R). HE.

tinguish two cellular types which have a different distribution in the vesicular wall (fig. 13): columnar cells with ovoid nuclei, radially oriented with respect to the lumen, and other smaller cells with spherical nuclei, located in the vicinity of the basal membrane. We call the cavities having these two levels of cells 'follicles', following the terminology of *Boya and Zamorano* [1975]. At each level, these layers were, respectively, distinguished as follicular layer, composed of columnar cells around the lumen, and the parafollicular layer, formed by the smaller cells situated between the follicular layer and the basal membrane.

The follicular and parafollicular layers correspond, respectively, to the ependymocytes and hipendymocytes of *Funkquist* [1912].

Stage 39 (13 Days)

From this stage until hatching, the pineal volume remains almost constant (fig. 21), showing only a very slow increase. Nevertheless, the parenchyma continues growing at a constant rate. The progressive growth of the parenchyma within a stationary pineal vo-

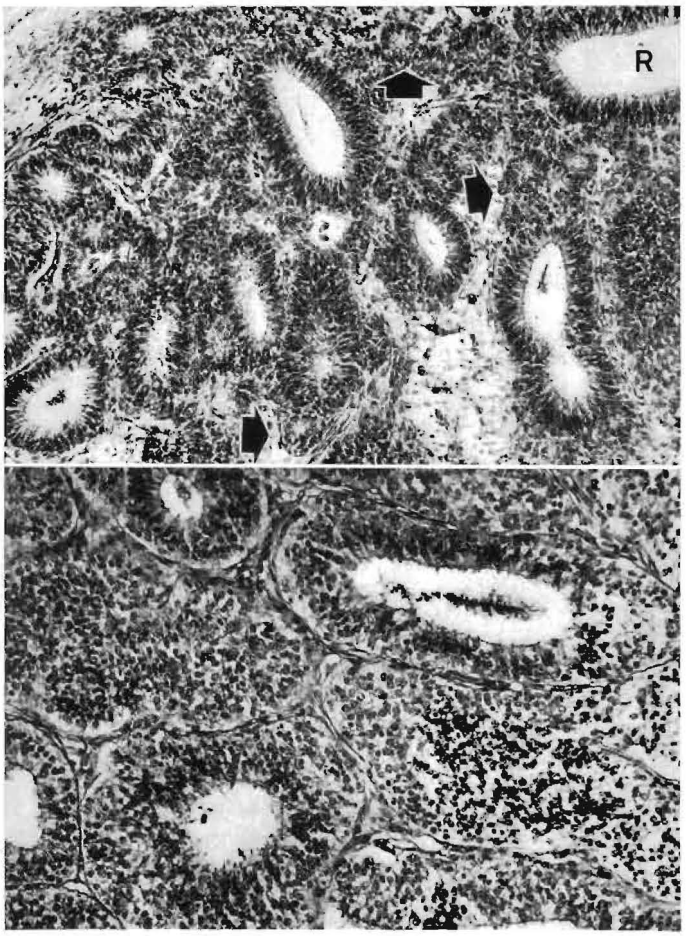

Fig. 15. Embryo of 13 days. Formation of cellular mammilliform projections in rosette formation (arrows) at the level of the parafollicular zone of the follicles. R = Recess. HE.

Fig. 16. Embryo of 17 days. Peripheral zone of the pineal gland. Less development of the follicles and greater quantity of interfollicular stroma. HE.

lume produces the compact aspect of the posthatching life (fig. 14). In the initial moment of the process of densification, we found the formation of cellular mammilliform projections on the walls of the follicles of the parafollicular cells. Examining these mammilliform projections under high-power magnification, they exhibit a rosette cellular arrangement which fills the future central lumen. The contours of the follicles are, therefore, more irregular and more uneven (fig. 15). At the same time, the interfollicular stroma is progressively diminishing due to the increase of the follicles. This stroma shows a larger quantity of vessels of small caliber and an ample lumen.

Stages 40 and 41 (14 and 15 Days)

The process of pineal densification, which was initiated during the last stage, continues. This process is more evident in the central zone of the gland. In effect, the medial sagittal sections show ample wall follicles due to an increase in the parafollicular level. The parafollicular layer is, therefore, responsible for the growth of follicles as well as for the pineal densification. The interfollicular conjunctive

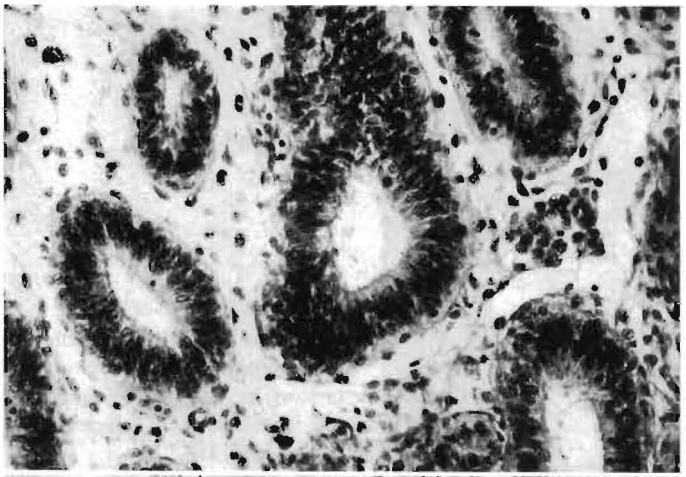

Fig. 17. Embryo of 17 days. Peripheral zone of the pineal gland. Slight development of the follicles and great quantity of interfollicular stroma. HE.

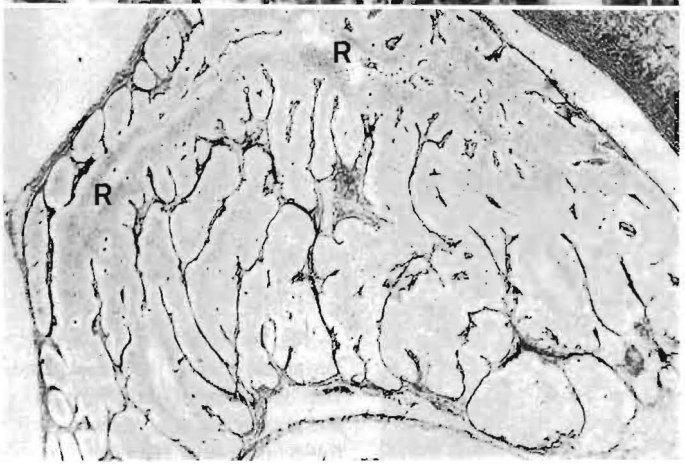

Fig. 18. Embryo of 21 days. Lobular disposition of pineal parenchyma. Note the existence of the recess (R) in the dorsal region of the gland. Technique of Gomori.

spaces continue their progressive reduction at a rate parallel to the growth of the follicles. The peripheral sections of the pineal gland constantly show follicles and vesicles separated by ample laminas of stroma which contain a large quantity of capillaries.

Stages 42 and 44 (16–18 Days)

The pineal gland is now practically compact (fig. 16). Only in the peripheral zone do we encounter vesicles and follicles as separated as in the earlier stages (fig. 17). The central zone shows large follicles with ample parafollicular layers which almost come in contact with each other. The stroma appears in the form of thin laminas, intercalated between these follicles. A diminishing of the caliber of the pineal cavities, especially of the recess and the large follicles, which stresses the compact aspect of the gland, is observed (fig. 16).

Stages 45 and 46 (19 Days to Hatching)

During the last days of embryonic development, the pineal gland of the chicken does not show any appreciable morphological variation; it presents, in sagittal sections, a triangu-

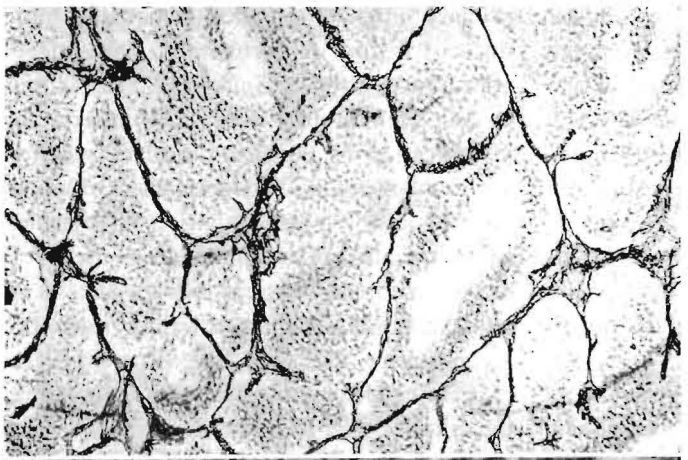

Fig. 19. Embryo of 21 days. Separation of the follicles by thin conjunctive partitions, made principally, or argyrophilic fibers. Technique of Gomori.

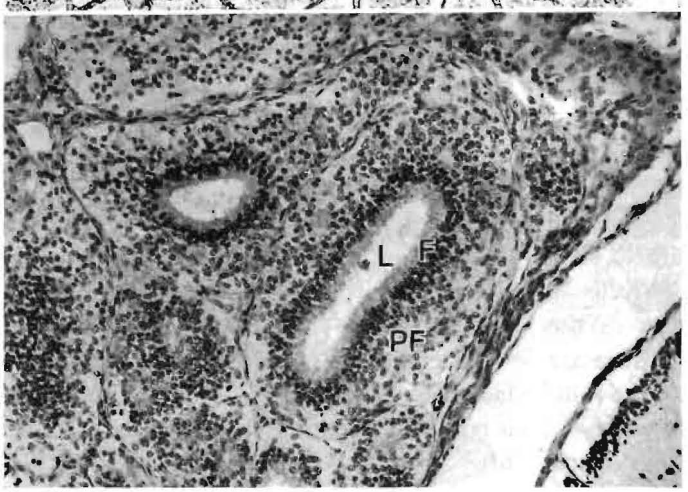

Fig. 20. Embryo of 21 days. Compact aspect of the pineal gland with typical follicular structure. Clear difference of the follicular zone (F) limiting the central lumen (L) and peripheral parafollicular (PF) zone. HE.

lar shape with an inferior vertex situated between the cerebral hemispheres and the cerebellum. The vertex is directed toward the roof of the third ventricle, dorsal to the region occupied by the choroid plexus. The base is oriented toward the cranial vault in which it is inserted by means of meningeal folds. The pineal is surrounded by a conjunctive capsule which is rich in collagenous fibers and fusiform cells. Outside this capsule, the profiles of large-diameter vessels are seen ['circus vasculosus' of *Beattie and Glenny,* 1966].

The pineal parenchyma is arranged following a clear follicular model in more advanced embryos. The argentic impregnation and the PAS techniques show the glandular lobules clearly, separated by fine conjunctive partitions (fig. 18). Notwithstanding, with the HE technique, a cavity is seen in the center of each lobule, for which, in reality, they are considered follicles (fig. 20). The distribution of the pineal follicles is not made at random but is strictly related to the pineal recess. This recess appears as a long cavity which, in the sagittal sections, is clearly displaced toward the dorsal surface of the gland (fig. 18). The

large follicles (which correspond to the first generation of vesicles which arises from the frontal wall of the recess) are arranged perpendicularly to the recess. In the anterior extremity of the gland, the parenchyma adopts a more compact aspect, losing the follicular model.

Separating the pineal follicles, we find conjunctive laminas which arise from the internal capsule. With the HE technique, the pineal stroma appears rich in capillaries and cells with dense fusiform nuclei, identifiable as fibroblastic cells. With argentic techniques, the existence of argyrophilic fibrils is demonstrated; these are more abundant in the vicinity of the basal membrane of the follicles and around the vessels (fig. 19).

The large pineal follicular cavities are surrounded by a well-defined follicular layer, composed of tall cells with ovoid nuclei arranged in two or three layers (fig. 20). The apical cytoplasm of the follicular cells is ample and eosinophilic. The limits of the lumen are very clear. In many cases there are filiform projections protruding into the follicular lumen. In the more mature embryos, the parafollicular layers are ample. An important fact is the presence, in the parafollicular layer, of cavities of variable size, but mainly small and surrounded by pinealocytes, radially arranged around the lumens. In the parafollicular layers, at high-power magnification, two types of cells can be distinguished by their nuclear characteristics: (a) large, clear global nuclei and (b) longer, denser nuclei of smaller caliber.

Discussion

The pineal outline appears early in the embryonic development of the chicken. The primary pineal evagination is formed during the first 3 days of incubation. The time of appearance, given by *Spiroff* [1958] at 48 h, is in our opinion too early. The criteria for identification of the first pineal evagination are fundamentally topographical; although one can observe a basal situation of the nuclei, a differentiation of the epithelium of the pineal outline is already noticed, which consists of a thickening of the anterior wall in relation to the posterior wall. The difference translates into the consequent evolution of the outline; the anterior wall gives rise to the major part of the vesicles and also to a major part of the diameter. The consequence of this different growth rate is the displacement in the dorsal direction of the pineal recess, which is visible in the more advanced embryos. The pineal gland of the chicken is derived fundamentally from the anterior surface of the pineal evagination. The posterior surface only gives rise to a small part of the gland situated dorsally to the recess.

After 5.5 days there is a definite frontal inclination of the axis of the pineal gland which is maintained until the 13th to 14th day, after which the gland is arranged vertically (fig. 21), because of the rapid growth of the neighboring structures, as *Spiroff* [1958] pointed out.

The pineal parenchyma is derived from the evagination of the roof of the third ventricle which is commonly called the pineal recess. At first, an ample communication exists between the pineal recess and the third ventricle. About the 12th to 14th day of development, the orifice of communication becomes strangulated, contrary to the affirmations of *Romieu and Jullien* [1942]. *Spiroff* [1958] affirmed that the closure of the communication can occur during the first 3 months after hatching. We think that the closure occurs close to the moment of hatching. In any case,

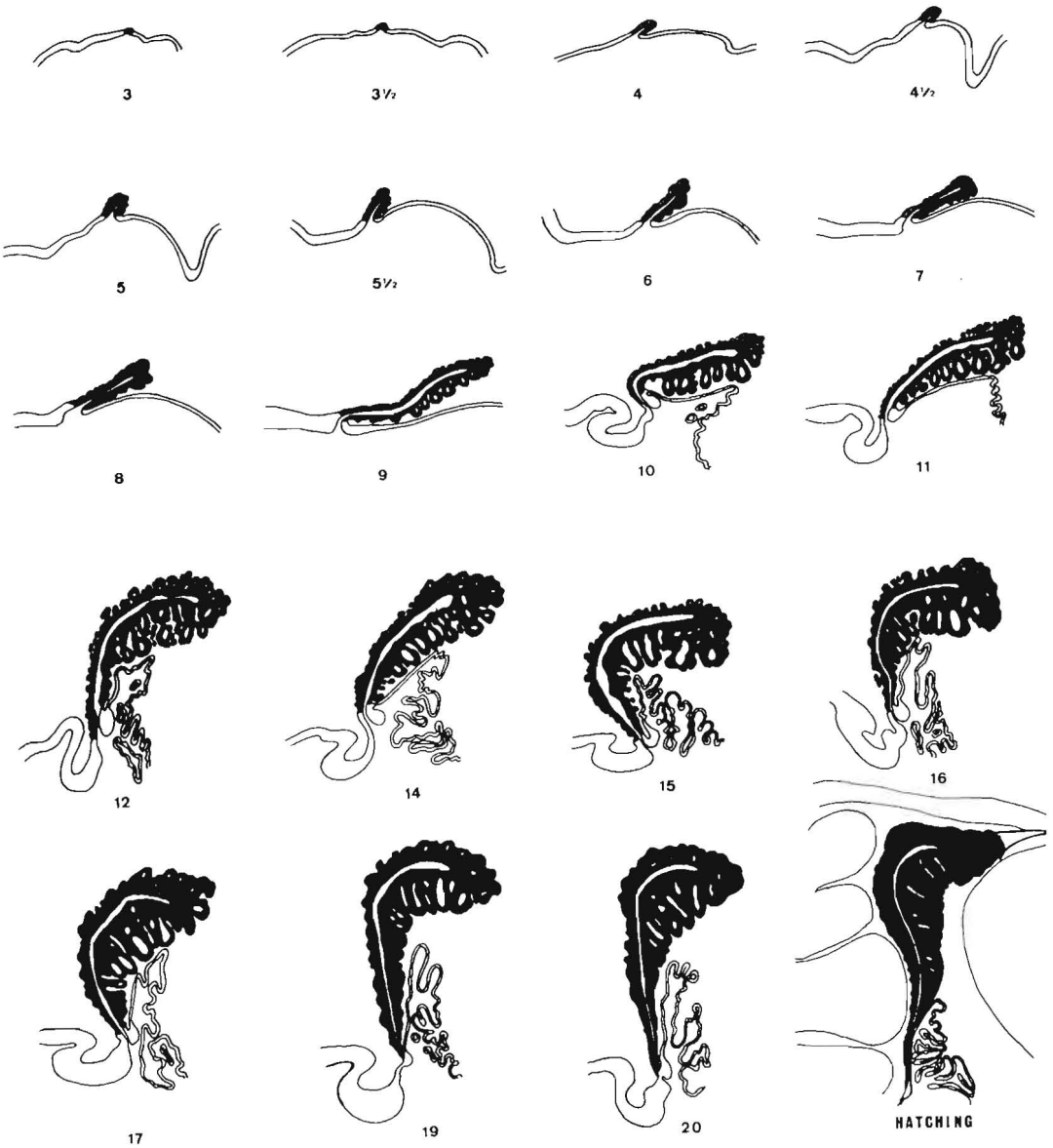

Fig. 21. Schema of the evolution of the pineal gland of the chicken during the embryonic development.

this problem is actually of secondary interest since it is not believed that there is a secretion of pineal products into the cephalorrhachidian fluid, according to the studies of *Romieu and Jullien* [1942] and *Wetzig* [1961].

The growth of the pineal outline, after the primary evagination, is produced by the formation of vesicles. The mechanism is always the same. The cells proliferate and form cellular mammilliform projections, which gives an irregular contour to the outline. Each mammiform projection shows a rosette cellular arrangement. Subsequently, the solid mammilliform projection transforms into a vesicle by the apparition of a central lumen. The vesicle transforms into a follicle by the thickening of its wall and reorganization of its cells into two cell layers. Three successive generations can be distinguished during the embryonic development of the pineal gland: (1) primary vesicles, formed on the wall of the pineal recess; (2) secondary vesicles, formed on the walls of the vesicles which were started by the evolution of the primary vesicles, and (3) tertiary vesicles, which arise from the spatial reorganization of the parafollicular pinealocytes that form cellular rosettes and, subsequently, vesicles and follicles.

Krabbe [1955] and *Spiroff* [1958] proposed that the pineal vesicles always form in a secondary manner. We think that the theory of *Romieu and Jullien* [1942], that the vesicles form in a primary manner by the evagination of the wall of the recess, must be abandoned. In no case did we encounter continuity between the vesicular lumen and the pineal recess.

The vascularization of the pineal gland is realized early in the embryonic development. After the 5.5th to the 6th day there is already a capillary plexus surrounding the future pineal gland. At 7 days, vessels exist in the intervesicular stroma. In relation to the peripheral capillary net, a densification of the stroma forms which gives rise to the capsule. All these data coincide, in general, with those of *Spiroff* [1958]. We think that the vascularization, appearance of the capsule, and densification of the pineal stroma form a part of the general process, which gives rise to the meninges, although the sequence of the process is earlier and more rapid at the level of the pineal gland.

The pineal gland grows rapidly until the 12th day of incubation, gaining in this stage a size similar to that which exists at the moment of hatching (fig. 21). The progressive and continuous increment of the parenchyma in a pineal volume which is practically stationary produces a gradual diminishing of the quantity of stroma and the apparition of a compact, solid aspect of the gland. The pineal glands of more mature embryos show a compact lobular aspect with thin laminas of stroma separating the lobules. Each lobule has a large central lumen; they are, therefore, considered follicles. Of the three models described by *Studnička* [1905], the pineal gland of the chicken before hatching pertains to the follicular type. These follicles are clearly arranged perpendicularly to the pineal recess and correspond to the first generation of vesicles which derive from the frontal surface of the outline. In the anterior extremity of the pineal, these large follicles are not seen. This zone derives from the point of the pineal evagination where abundant but small-diameter vesicles are formed. Behind the recess, some vesicles which are derived from the posterior surface of the pineal evagination are seen.

The pineal follicles have in their walls two well-defined zones or levels named the follicular and parafollicular zones, following the terminology of *Boya and Zamorano* [1975]. In

each of the layers, two types of cells can be distinguished with the optical microscope. We consider, therefore, as did *Boya and Zamorano* [1975], that the follicular and parafollicular levels represent two models of organization of the pinealocytes and not two types of cells, namely ependymocytes and hipendymocytes, as had been thought since *Studnička* [1905].

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