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Ultrastructural study of the embryonic development of the pineal gland of the chicken *(Gallus gallus)*

J. Calvo and J. Boya

Department of Histology and General Embryology, Faculty of Medicine, University Complutense, Madrid, Spain

Key words. Pineal · Chicken · Embryonic development · Ultrastructure

Abstract. The authors studied the embryonic development of the pineal gland of the chicken with the electron microscope. The denomination of 'pinealoblasts' was given to the undifferentiated cells which form the primitive pineal outline. In the wall of the pineal cavities, the follicular and parafollicular zones were distinguished; these are formed by type A and type B pinealocytes, the B type being much more abundant. The degenerated cells are constant in the pineal throughout its embryonic development, but much more abundant in the early phases.

Introduction

Until now no studies have been published concerning the ultrastructure of the pineal gland of the chicken, during its embryonic development, even though it is an animal commonly used in embryological investigations. Only a few studies using the optical microscope have been published, mainly at the beginning of the century [*Cameron,* 1903; *Studnicka,* 1905; *Funkquist,* 1912], but these have little value from the morphological point of view. In more recent years we have only the studies of *Krabbe* [1955], *Spiroff* [1958] and *Calvo and Boya* [1977]. *Collin* [1966a, b], with the electron microscope, cited the moment of apparition of the pineal cellular types (approximately 12 days) in the embryonic development of the magpie.

In a previous study, we described the embryonic development of the pineal of the chicken with the optical microscope [*Calvo and Boya,* 1977]. In the present study we studied the ultrastructure of the pineal gland of the chicken during its embryonic development.

Materials and methods

We used, for our study, the pineals of chick embryos incubated under controlled conditions. We took samples every 24 h after the first 5 days of incubation until the moment of hatching.

The pineals were fixed in 3% glutaraldehyde in phosphate buffer or in 0.1 *M* osmium dichromate (Dalton). In the first case, the pieces were fixed, afterwards, in osmium tetroxide in the corresponding buffer. The pieces were then included in Vestopal W after previous dehydration in acetone. The double-fine cuts, obtained with an LKB ultramicrotome, were double-contrasted with uranyl acetate and lead citrate. The examination of the specimens was made with a Philips EM 201 microscope.

Results

As was stated earlier, the present study refers only to the ultrastructure of the pineal of

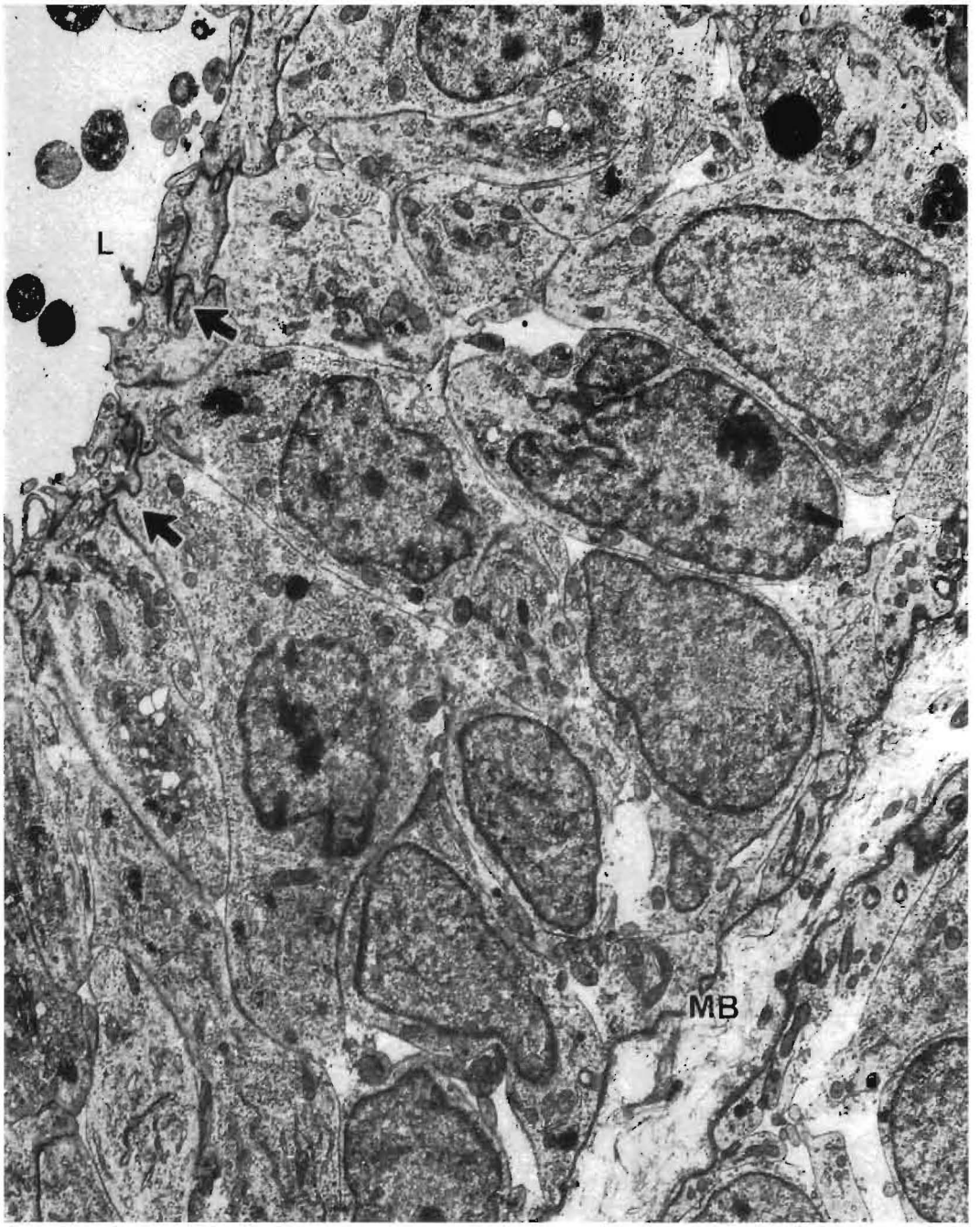

Fig. 1. 5-day-old embryo. Wall of the pineal evagination in which two lines of nuclei, one of them very close to the basal membrane (MB), can be distinguished. The pinealocytes on the border with the lumen of the cavity (L) demonstrate abundant mechanisms of union (arrows). × 5,000.

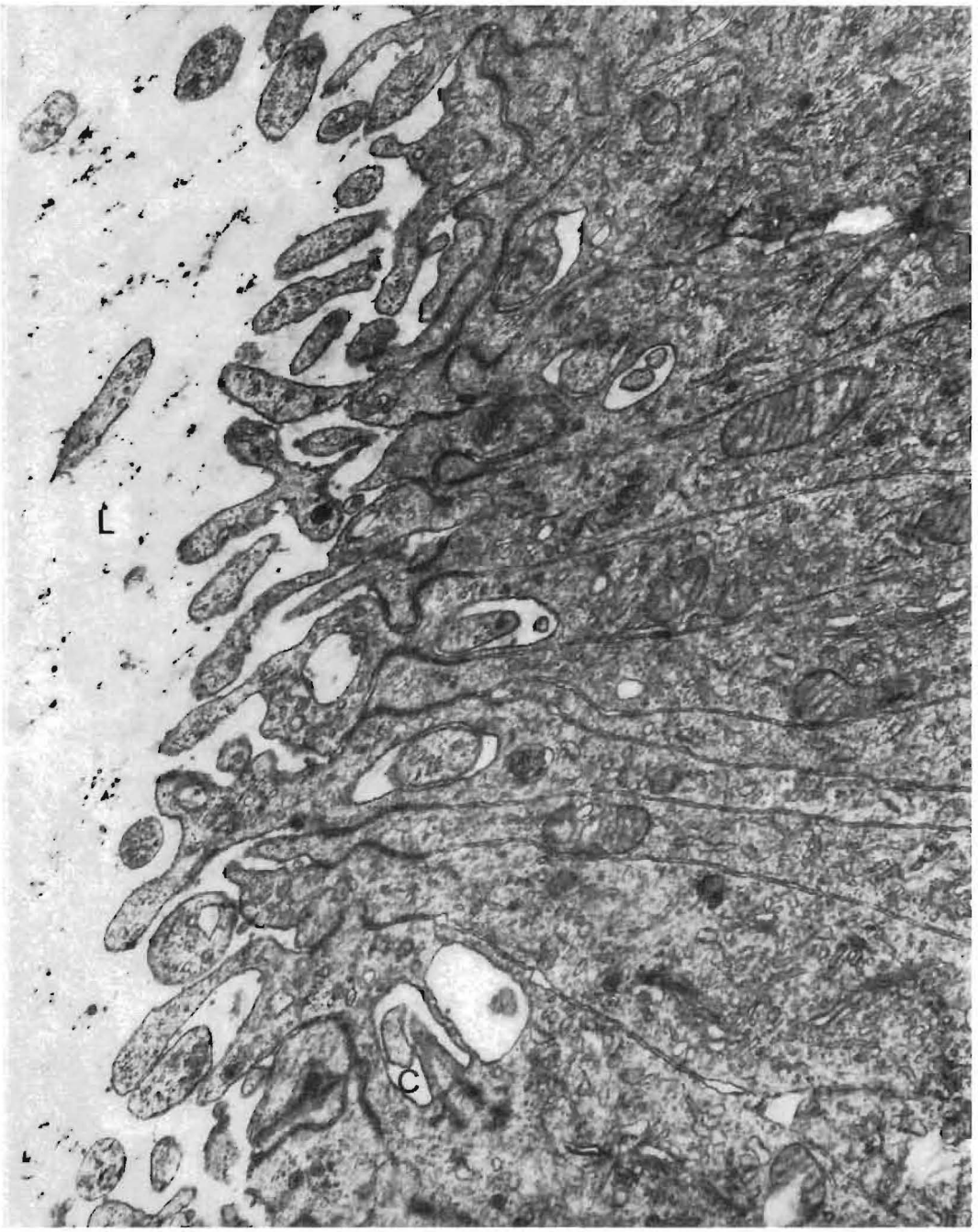

Fig. 2. 6-day-old embryo. Supranuclear cytoplasm of the pinealocytes which sends finger-like prolongations toward the lumen of the cavity (L). Note the existence of cilia (C) with their corresponding basal corpuscle. × 10,000.

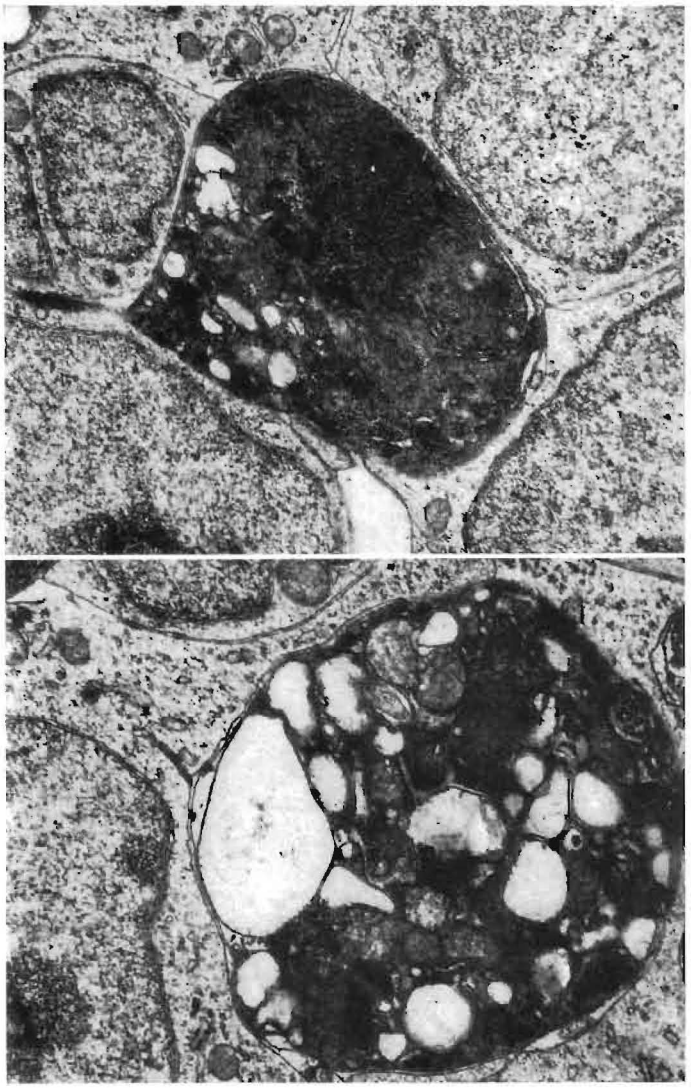

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Fig. 3, 4. 6-day-old embryo. Degenerated cells. Existence of remains of cytoplasmic organelles. **3** × 15,000. **4** × 20,000.

7–8 days of development

The pineal outline shows numerous round vesicles with a very small central lumen (fig. 5). The ample intercellular spaces in the covering epithelium continue to exist. In the large vesicular formations, the epithelial cells are arranged in two lines located in the middle third and the basal third (fig. 5). This stratification is only apparent, all the cells extend from the basal membrane to the vesicular lumen.

The pineal vesicles are separated by ample spaces which are occupied by a lax stroma which contains star-shaped cells having a mesenchymatous aspect and thin conjunctive

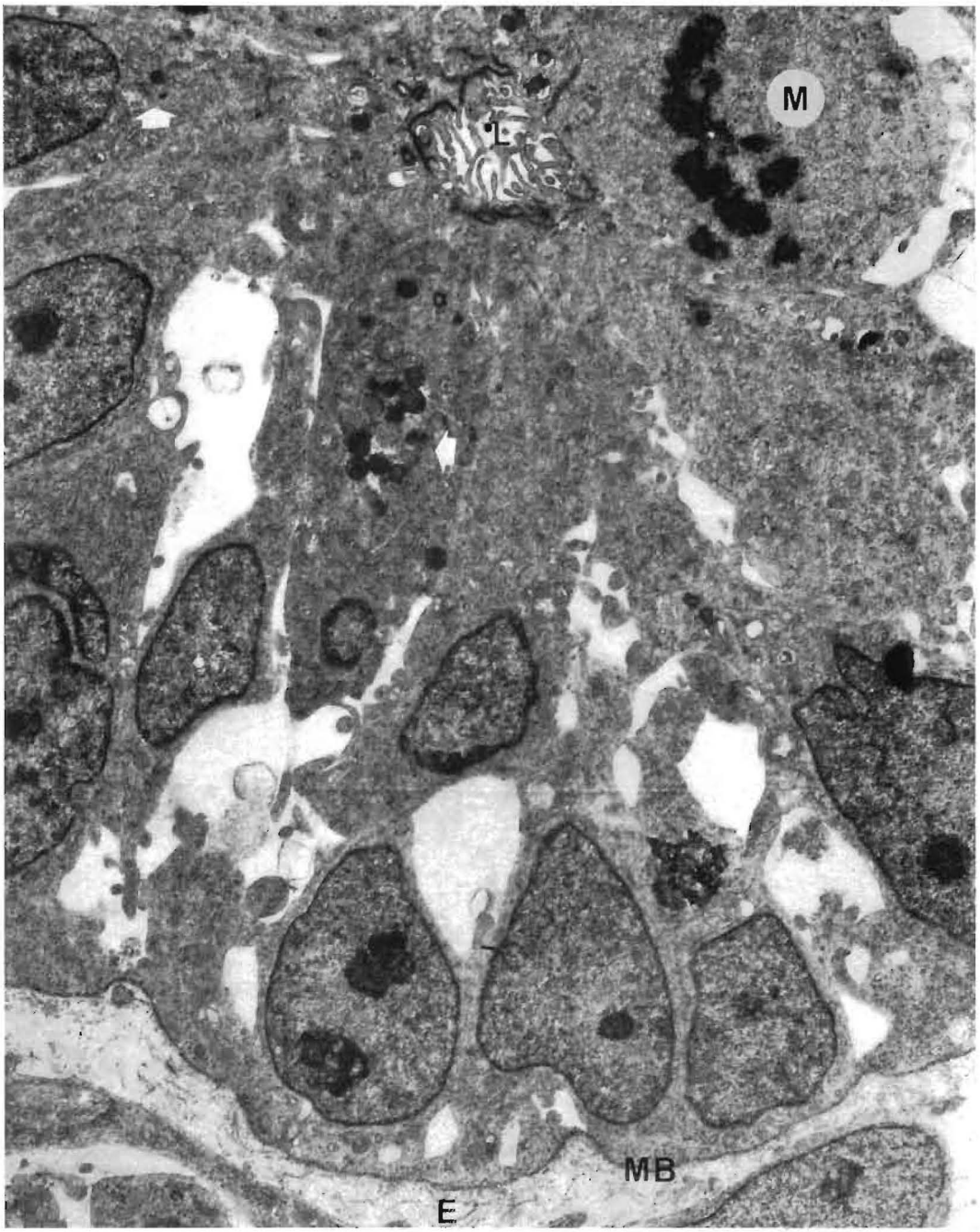


Fig. 5. 7-day-old embryo. Pineal vesicle with small-caliber central cavity (L) toward which the pinealocytes direct finger-like prolongations. There are abundant mechanisms of union, practically at the limit of the cavity. Lipid bodies in the supranuclear cytoplasm of the pinealocytes (arrows). A cell in mitosis (M) in the

fibrils; in relation to the basal membranes, these fibrils start to show transversal striations. We encountered capillaries with an immature aspect in the pineal stroma after 7 days of development. We also noticed a peripheral densification that leads to the apparition of a capsule.

The basal zone of the pineal vesicles is formed by cell bodies (fig. 5) and striated prolongations which rest on the basal membrane, thus forming a continuous layer. The content of the basal prolongation is similar to that described in the last period. There are abundant intracellular dense cells and bodies. The nuclei of the pinealocytes have a dispersed chromatin and one or two evident nucleoli. There are numerous mitotic figures in the vicinity of the vesicular lumen (fig. 5). The basal cytoplasm of the mitotic cells is retracted which accounts for the high location of the mitotic images on the vesicular wall. The cells in division continue to demonstrate unions with the neighbouring cells. The apical cytoplasm is more abundant than in the last phase. We observed the following differences: small, round, lipid drops which are visible with glutaraldehyde fixation (with osmium dichromate fixation they appear star-shaped) after 7 days of embryonic development. We also found microtubules after 8 days but in very small quantity. The basal corpuscles of the cilia continue in their low position, always below the union complexes. Only occasionally did we find ciliar outlines in the vesicular lumen, which indicates the shortness of the cilia.

vicinity of the lumen. The vesicle is separated from the pineal stroma (E) by the basal membrane (MB). ×4,500.

9–10 days of development

The pineal still has ample cavities with thick cellular walls and also small vesicles, generally located in a more peripheral position. The stroma is more cellular and has a more developed capillary plexus than in the last phase. The cells of the stroma have ample cytoplasms which are slightly electron-dense and rich in organelles which include the rough reticulum, mitochondria, round lipid drops and abundant microfilaments (fig. 6). In the intervesicular stroma the cells have star-shaped or fusiform contours. The peripheral densification of the stroma, which began in the last phase, has given rise to the development; the pineal capsule consists of various laminas of flattened cells superposed among themselves. Between the cellular laminas remains an interstice rich in fine fibers in which capillaries are frequently seen (fig. 7).

In this phase, in the walls of the pineal cavities of larger caliber, we begin to distinguish two cellular layers: tall cells, which are radially oriented, delimiting the lumen; and cells of smaller caliber with a polygonal contour, arranged without precise order, between the anterior layer and the basal membrane (fig. 8). Following the terminology of *Boya and Zamorano* [1975], we designate these layers 'follicular' and 'parafollicular', respectively. The pineal cavities in whose walls these two layers are found we call 'follicles'.

The parafollicular zone, although slightly developed, contains small cells with ovoid or spherical nuclei, poor in organelles and having small short prolongations. Some head toward the basal membrane where they end in a dilation. We also saw cell bodies leaning on the basal membrane. The abundance and amplitude of the intercellular spaces are characteristic of the parafollicular layer. After 9–10 days of incubation, the degenerated cells in the

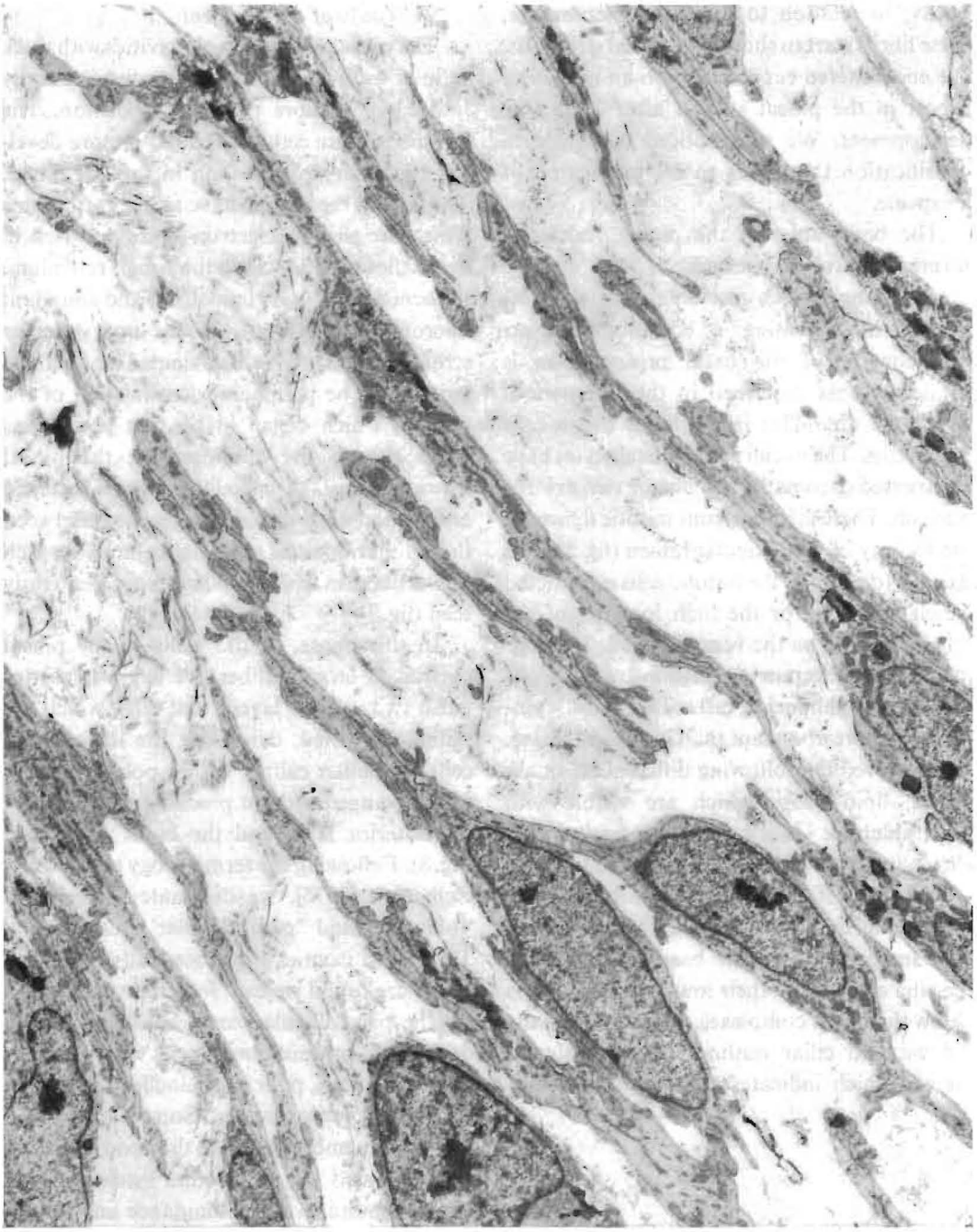


Fig. 6. 10-day-old embryo. Pineal capsule formed by cytoplasmic laminas from fibroblastic cells between which there is a small quantity of collagenous fibrils. × 3,000.

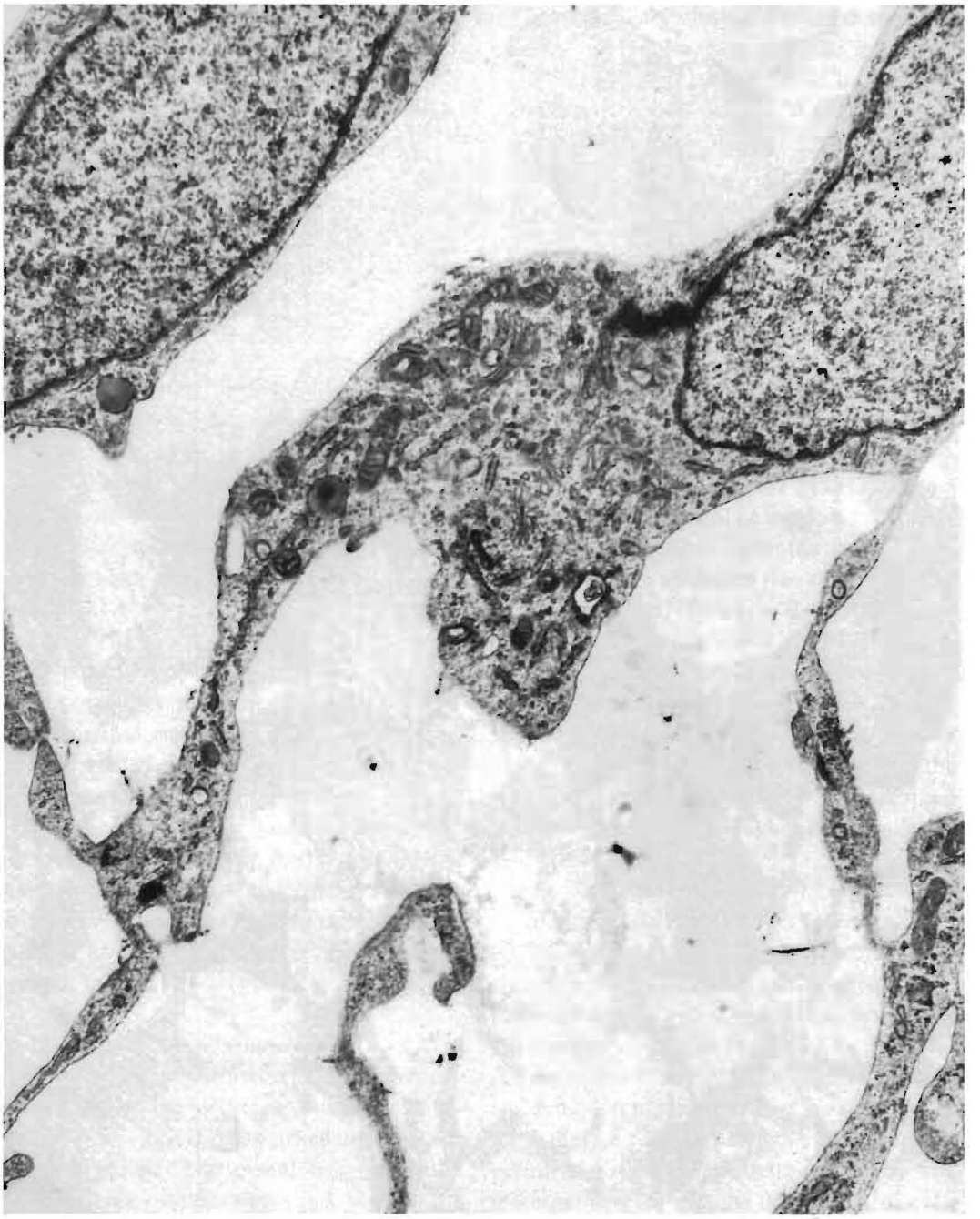


Fig. 7. 10-day-old embryo. Fragment of fibroblastic cells, rich in organelles, from the pineal capsule. × 7,000.

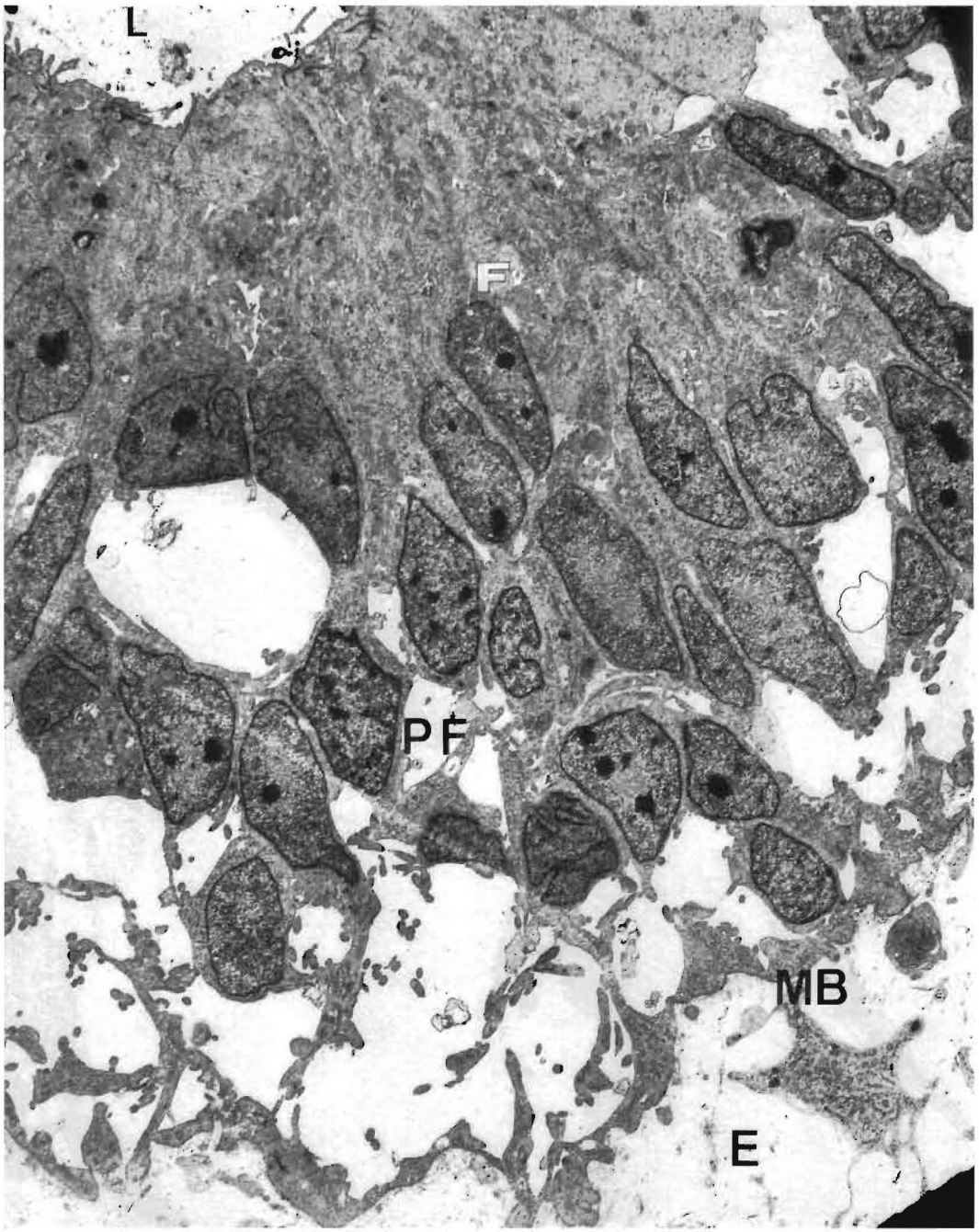


Fig. 8. 11-day-old embryo. Portion of the follicular wall with the compact follicular zone (F) which limits the cavity (L) and the parafollicular zone (PF) with ample intercellular spaces. This last zone leans on the basal membrane (MB) which separates the follicle from the pineal stroma (E). × 2,000.

pineal are exclusively found in the parafollicular zone.

In this phase of embryonic development the differentiation of a second cell type, which in the older embryos is converted into the principal pinealocyte, begins. We call these cells B pinealocytes, following the denomination proposed by *Boya and Zamorano* [1975]. The rest of the pineal cells constitute probably, in this phase, a heterogeneous population with undifferentiated cells or cells in the process of differentiation.

The first B pinealocytes appear as small cells with dense nuclei and a thin electron-dense cytoplasm.

The follicular layer is composed of long cells, in scanty groups, without the ample intercellular spaces which exist in the parafollicular layer. The nuclei are located in the lower third of the follicular layer. The two cell types described in the parafollicular layer are observed even when the proportion of the second or B type pinealocyte is minimal. The ultrastructural characteristics are identical to those described earlier, emphasizing the small size of the cells. The rest of the follicular pinealocytes present few variations, pointing out the greater amplitude of the apical cytoplasm which has many small lipid drops. The luminal projections are laminar or bulbar and contain abundant ribosomes and covered vesicles.

11–12 days of development

There are few ultrastructural differences with respect to the last stage, except the habitual growth, which is very marked in this phase of development. The pineal has numerous follicular-type cavities with thick walls, with two cellular types and vesicular cavities of smaller caliber, thinner walls and abundant intercellular spaces.

In the follicles, the parafollicular layer has evolved little. It appears as a thin layer with small cells separated by ample intercellular spaces. As a difference, we found the greater longitude of the prolongations of the parafollicular pinealocytes. The follicular zone has a thickness and aspect similar to that found in the last stage.

The B pinealocytes are abundant. We also note their increase in size. The ultrastructural aspect is similar to the last described stage. The nucleus has a lax chromatin situated in a dense nucleoplasm. We did see thick chromatinic masses. Generally, they had one or two nucleoli. Invaginations of the nuclear membrane begin to appear in this phase, although they are only slight. The cytoplasm is also dense in comparison with the neighboring cells. They have abundant free ribosomes and rough reticulum. There is a greater mitochondrial richness in the B pinealocyte. It is important to note that, in this phase and to a lesser degree in the last phase, terminal drumsticks and ciliar prolongations begin to appear (fig. 9, 10), although in small quantity, in the follicular lumen. We did not find terminal drumsticks in cavities lacking the B pinealocytes.

13 days of development

The most important ultrastructural characteristic of the pineal of the chicken in this phase of embryonic development is the appearance of cavities in formation in the parafollicular zone (fig. 11). In the 12-day-old embryos, a few examples may be seen. At 13 days they are now sufficiently abundant to permit a more complete description. The formational cavities may be distinguished, even at low power, by the mechanisms of union in the parafollicular layer (fig. 11, 12). In the earliest phase of formation of one of these

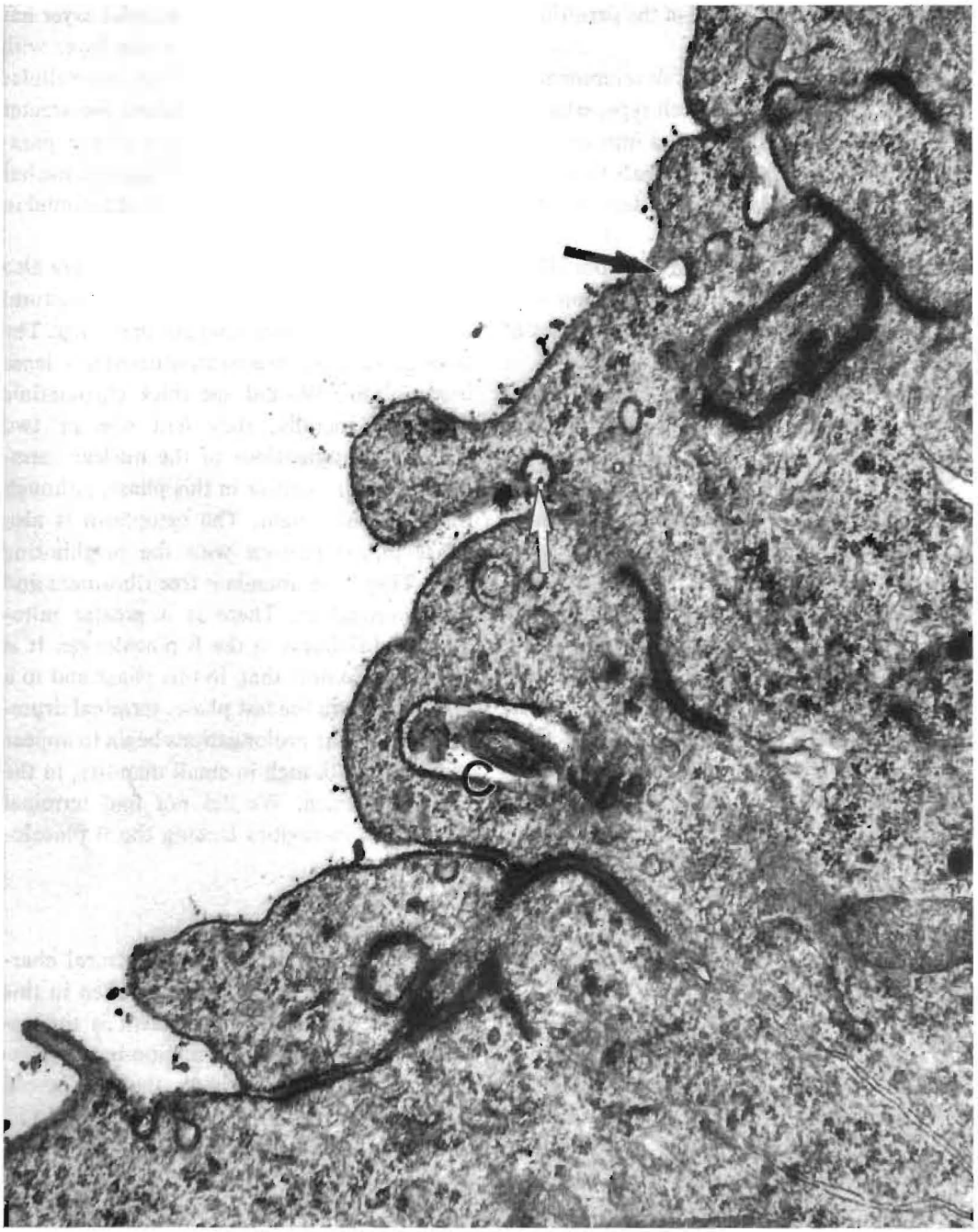
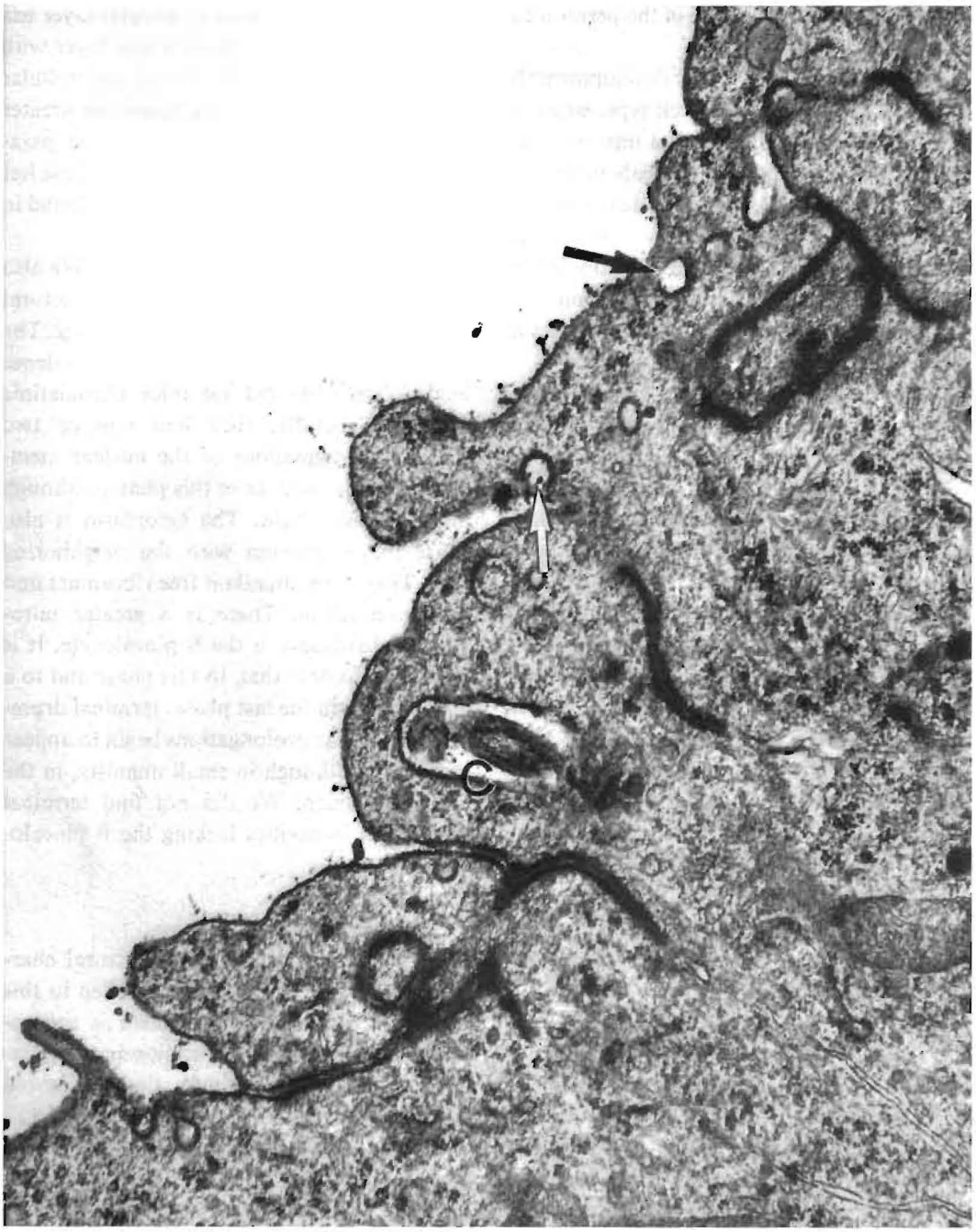


Fig. 9. 12-day-old embryo. Apical cytoplasm of the pinealocytes with bulbar projections. Note the existence of short transversal cilia (C) and the existence of vesicles, some of them opening to the follicular lumen (arrows). × 15,000.



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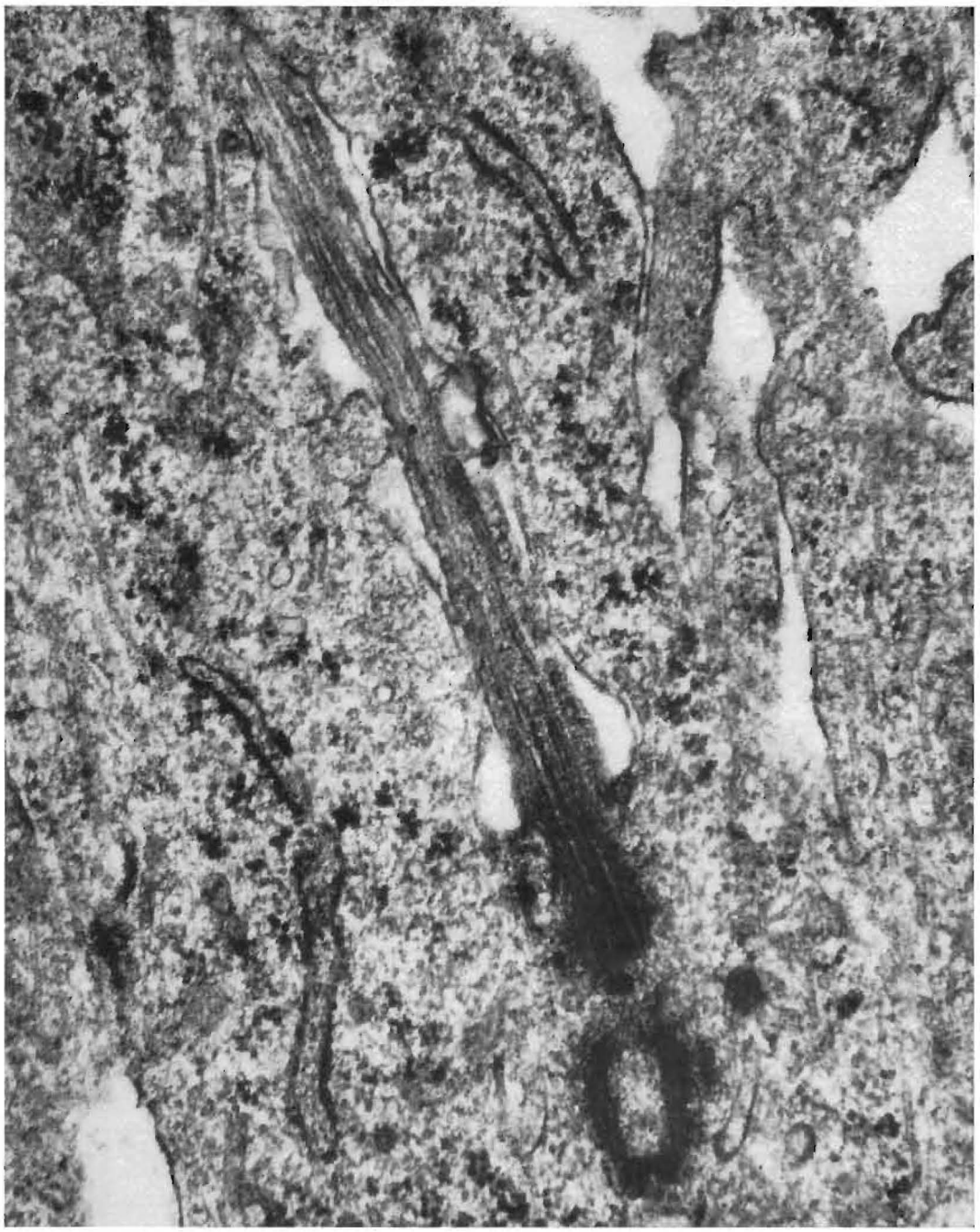

Fig. 10. 12-day-old embryo. Ciliar prolongation with its basal corpuscle in the apical cytoplasm of a follicular pinealocyte. × 30,000.

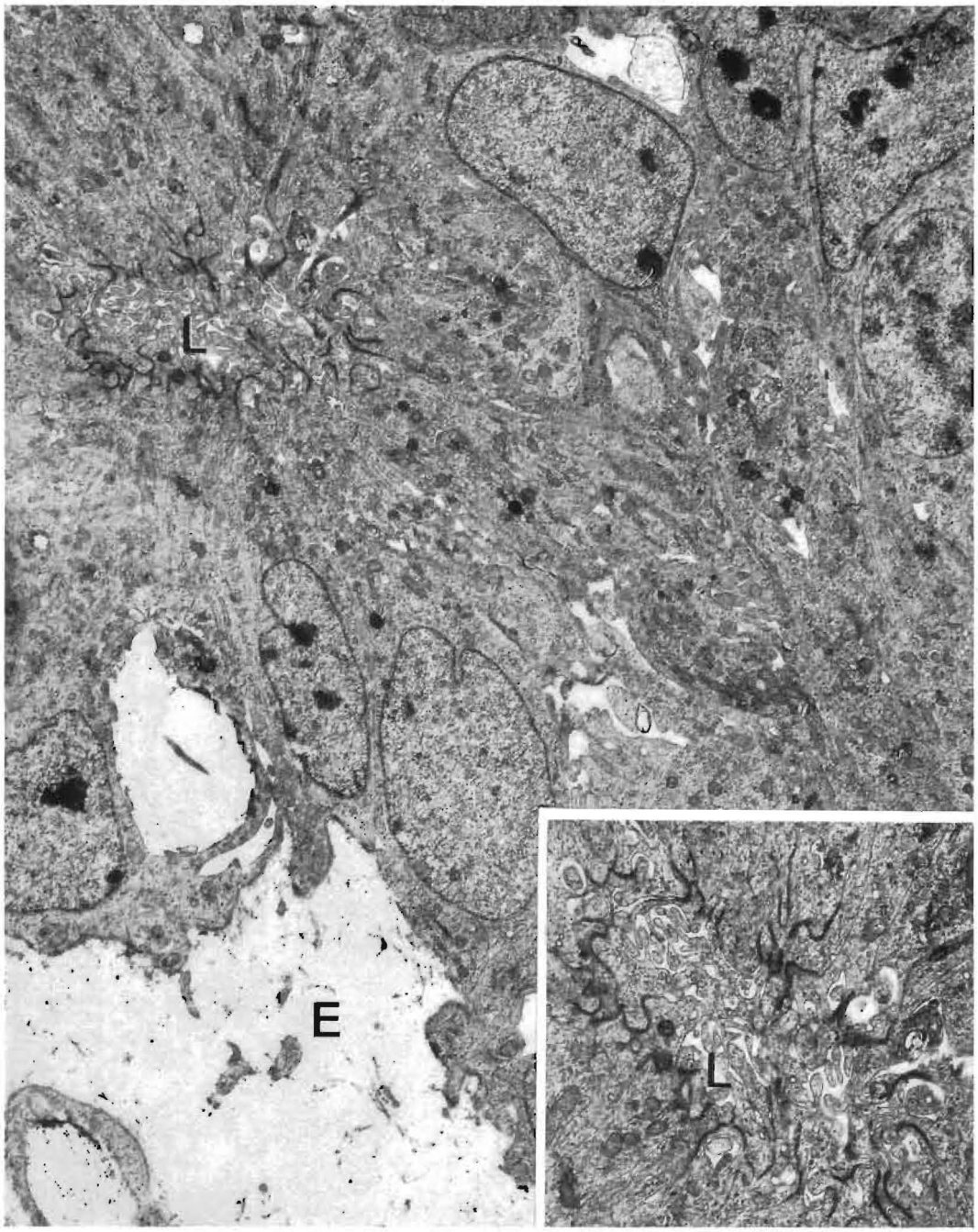

Fig. 11. 13-day-old embryo. Parafollicular zone with the formation of a follicular lumen (L) in the vicinity of the stroma (E). The cavity is very small and a large part of it is filled by finger-like prolongations of the pinealocytes. × 3,000.

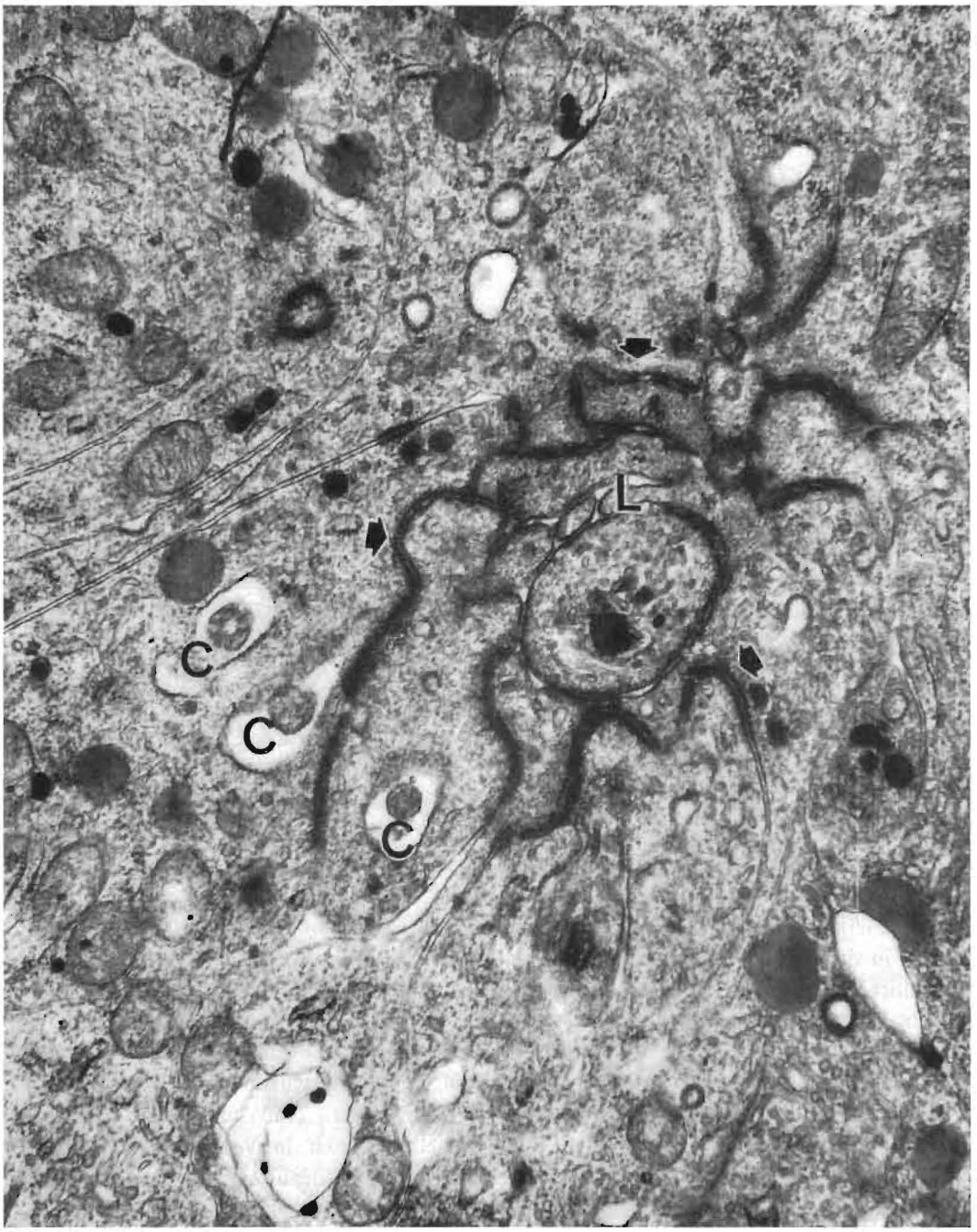

Fig. 12. 13-day-old embryo. Parafollicular zone. Formational follicle in which there is a great development of the mechanisms of union between the cells (arrows). Note the existence of ciliar processes (C) and lipid drops in the vicinity of the lumen (L). × 15,000.

cavities we find various cellular formations which join at one point; at this point we see the mechanism of union. The cellular prolongations are ample but always smaller than the apical cytoplasm of the follicular pinealocytes. They contain many organelles with lipid drops and ciliar prolongations present; the basal corpuscle is deeply located (fig. 12), which appears in the initial phases even before the apparition of the lumen. Afterwards, the central lumen appears, minimal in size and almost totally occupied by apical finger-like prolongations of the pinealocytes (fig. 11). Invariably, all the visible mitoses in the parafollicular zone are associated with a cavity in formation. These mitoses are responsible for the amplification of the central lumen. There are cavities in formation in the parafollicular zone, in progressively growing numbers, from the 13th day of development until the moment of hatching.

14–15 days of development

At 14–15 days of development, the B pinealocytes acquire the morphological characteristic of this type of cell, which develops more in later phases. The proportion of these cells has increased considerably. In the parafollicular zone they appear as cells with ample cytoplasm, and ovoid nuclei presenting invaginations of the nuclear membrane which are located in the superior pole, oriented toward the follicular lumen. Immediately above the nucleus, we find a region rich in rough reticulum and a large lipid drop (usually only one) which has a laminar aspect in its periphery. The rest of the apical cytoplasm contains a poorly developed Golgi system, mitochondria and microtubules. There are very small dense bodies with very polymorphous contours (elongated forms, drumstick forms, spherical forms, etc.). At the point of union, the B pinealocyte becomes narrow or forms a 'neck' in which we find unions of the zonula adherens type and many microtubules. The neck continues with a dilation of 'terminal drumstick' located in the follicular lumen. In many cases a short cilium, whose basal corpuscle is in the terminal drumstick, can be seen.

16–17 days of development

All the evolutionary lines initiated in earlier phases continue their development. In the follicular zone the increase in the number of B pinealocytes is very evident as is the morphological maturation of these cells (fig. 14). However, the most evident changes are located in the parafollicular zone, which appears to be formed by cells with a more ample cytoplasm than in earlier phases. This causes a decrease in the intercellular spaces, characteristic of the parafollicular zone. Perhaps the most important characteristic of this period is the apparition of thin cellular prolongations, joined in small bundles (fig. 15) located between the parafollicular pinealocytes which head toward the basal membrane. Some of the prolongations contain dense granules, which are sometimes separated from the enveloping membrane by a clear halo. These correspond to prolongations of the B pinealocytes.

18–21 days of development

There are few ultrastructural variations in the pineal of the chicken after 18 days of incubation. Around the parenchyma there is a capsule formed by cellular layers separated by collagen fibers which are arranged in interwoven layers. Thin conjunctive partitions, which are rich in vessels, part from this capsule and locate themselves between the follicles and vesicles. These partitions have more abundant fibroblasts and collagen fibers than earlier states. We did not find nerve fibers in the partitions before hatching.

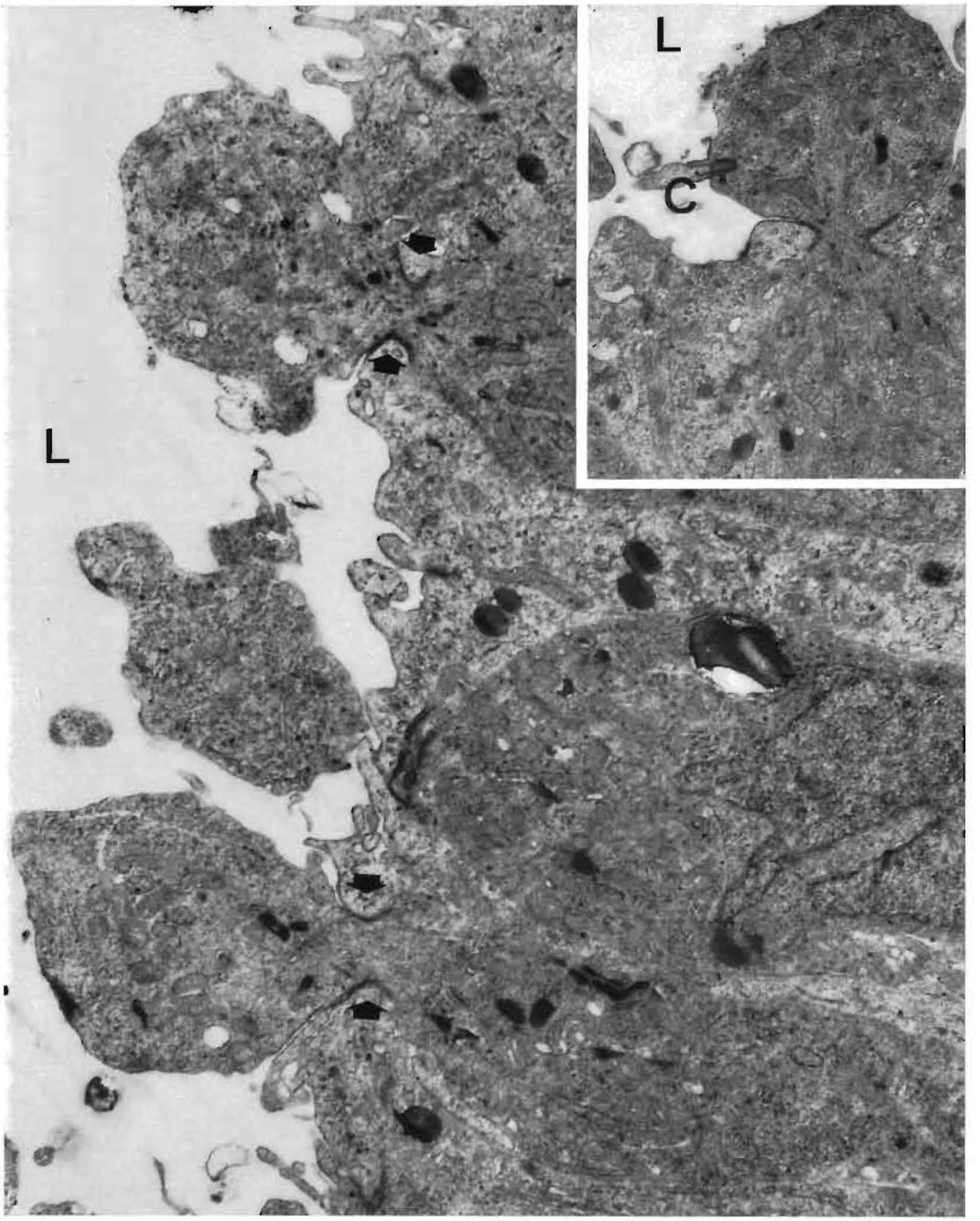

Fig. 13. 15-day-old embryo. B pinealocytes with bulbar prolongations in the follicular lumen (L). Ciliar formation (C) in one of these prolongations (panel) and abundant microtubules in the neck, at the level of which are the mechanisms of union (arrows). × 7,000.

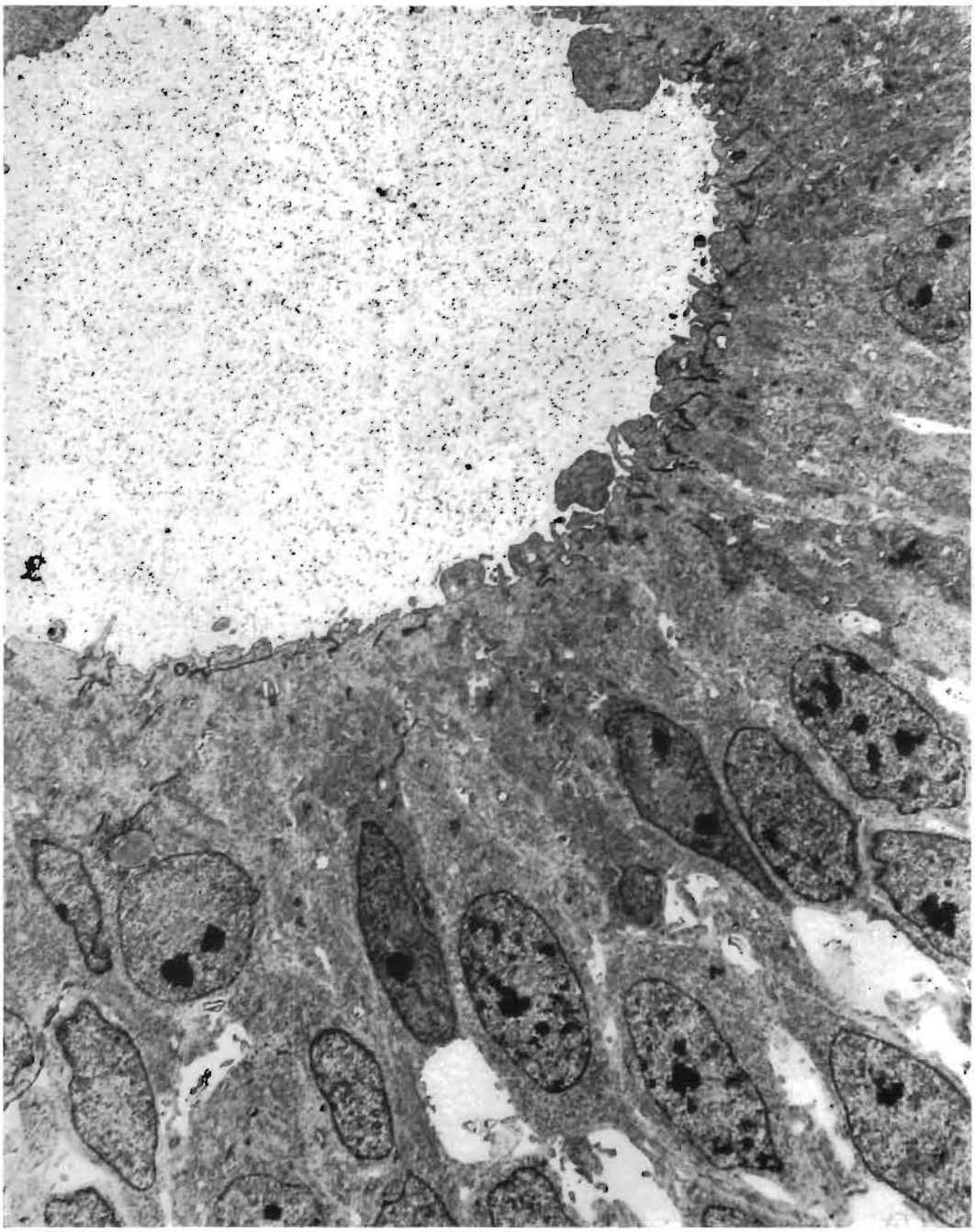

Fig. 14. 17-day-old embryo. Follicular zone with predominance of B pinealocytes. × 2,000.

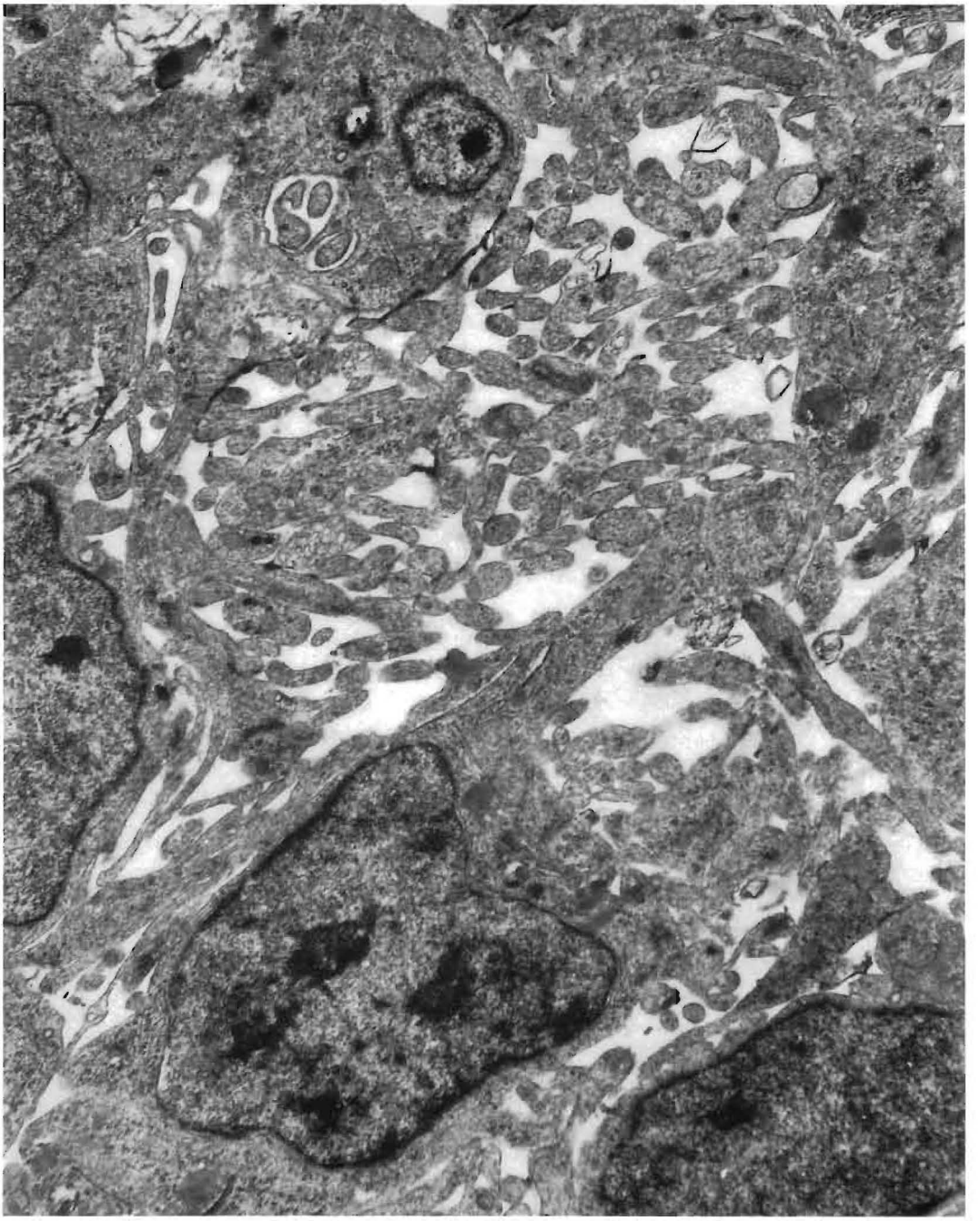

Fig. 15. 17-day-old embryo. Area rich in cellular prolongations in the parafollicular zone. × 7,000.

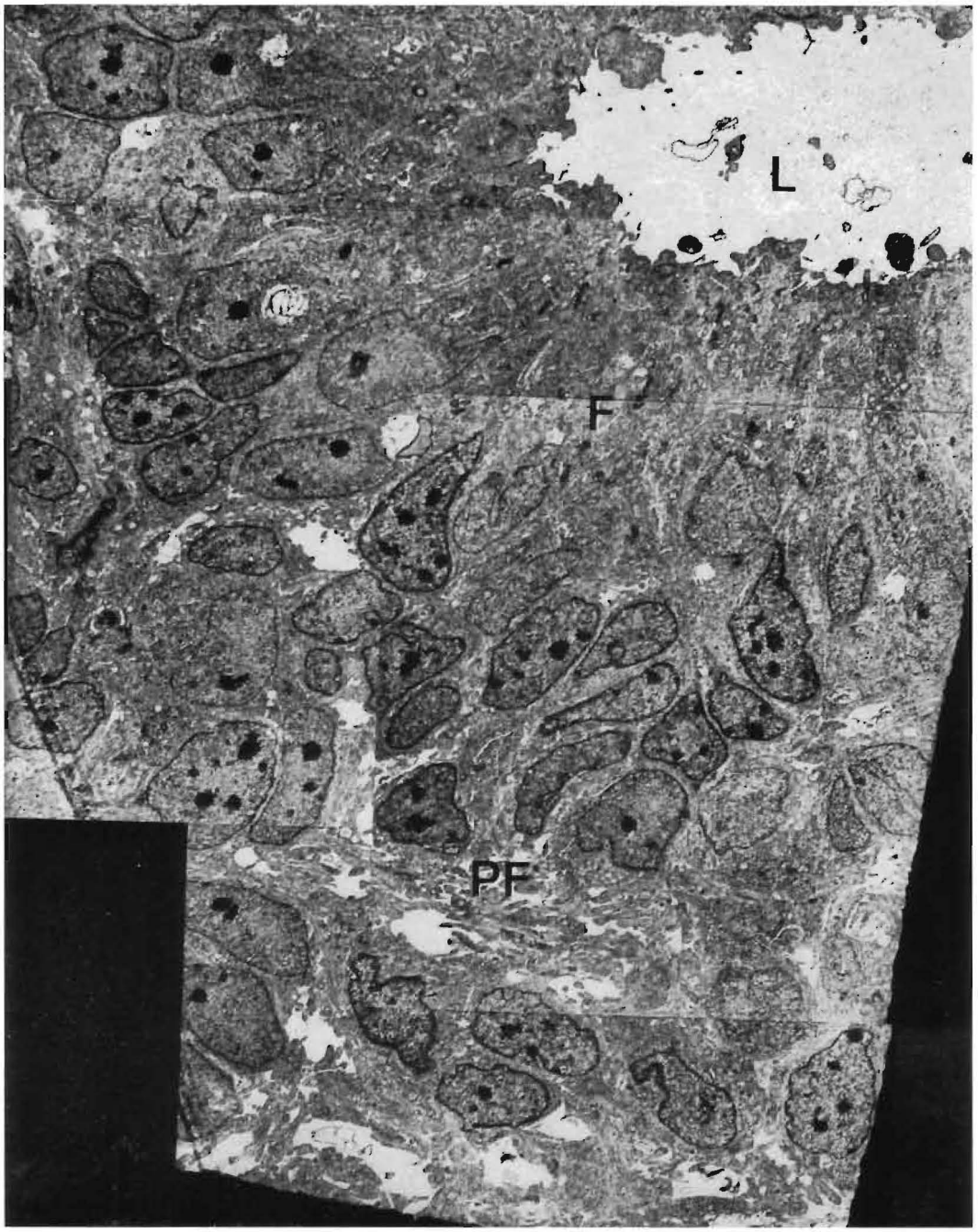

Fig. 16. 20-day-old embryo. Follicular wall in which the follicular zone (F) limiting the central lumen (L) and the parafollicular zone (PF) can be observed. Composition.

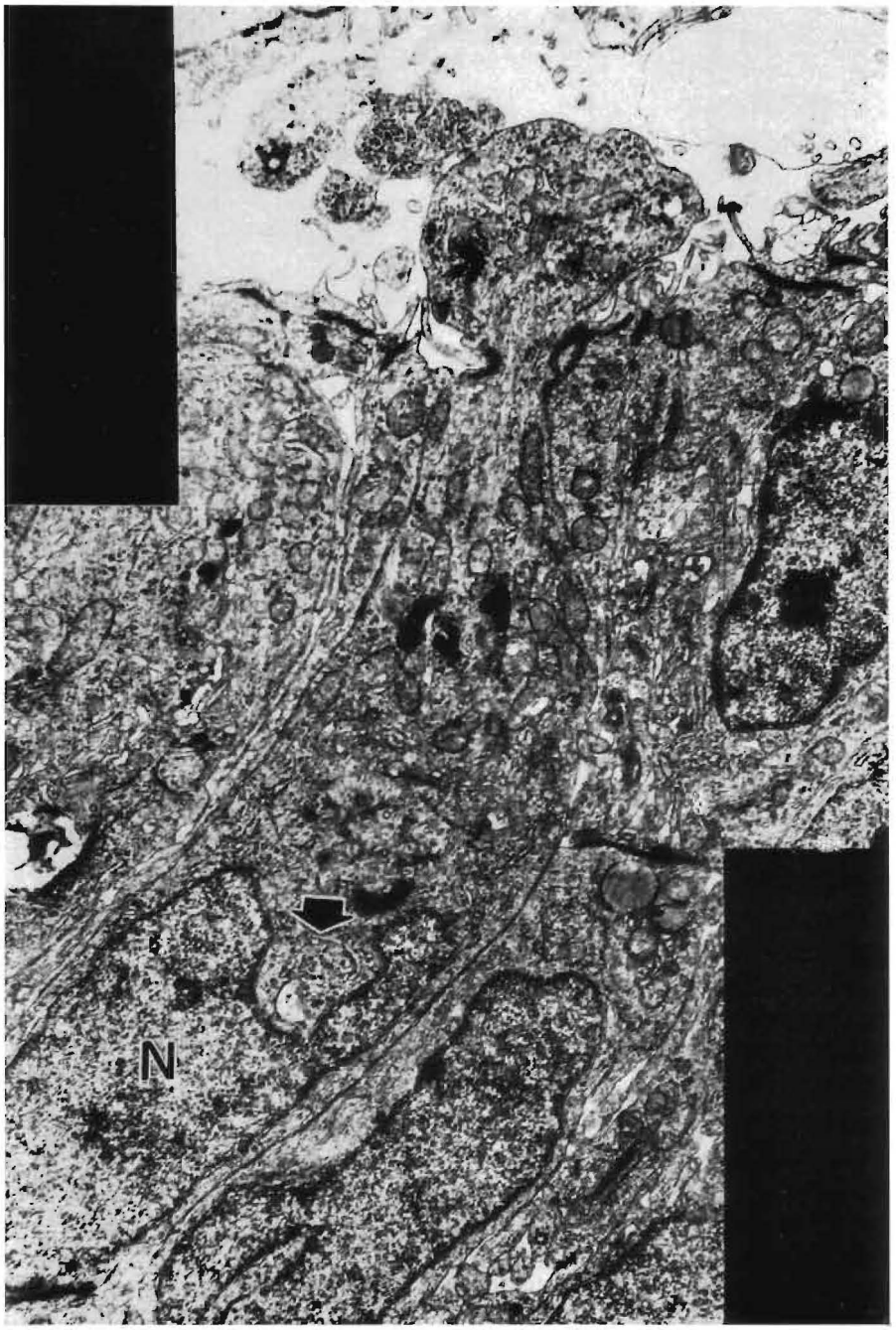

Fig. 17. 20-day-old embryo. B pinealocyte of the follicular wall. Nucleus (N) with furrow in the superior pole (arrow). The apical cytoplasm is rich in organelles and continues with the terminal drumstick, at the level of which exist mechanisms of union. Composition.

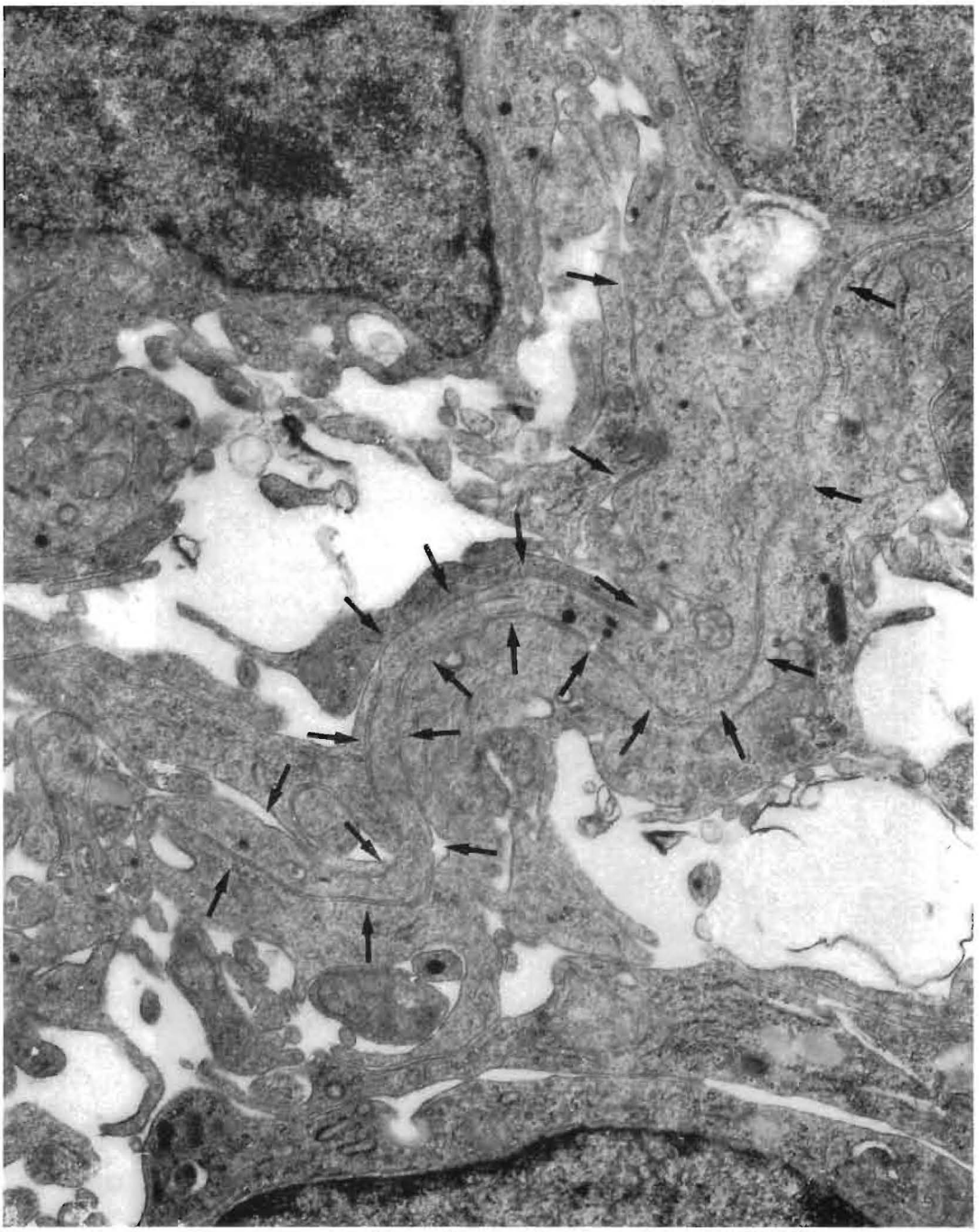

Fig. 18. 21-day-old embryo. Limit of the follicular and parafollicular zones. Basal prolongation of a B pinealocyte with dense grains in its interior. × 10,000.

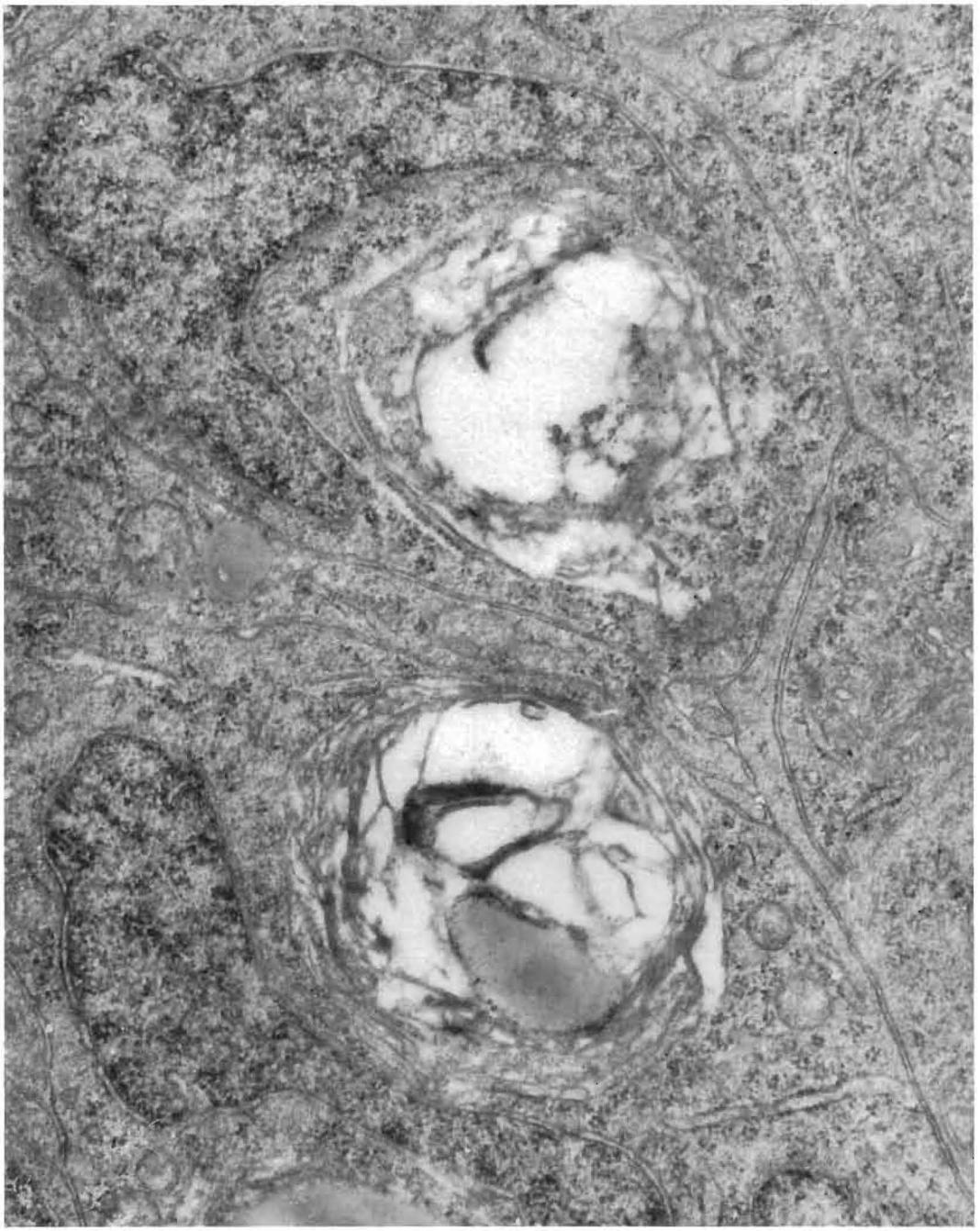

Fig. 19. 19-day-old embryo. B pinealocytes of the follicular zone. Laminar lipids. × 15,000.

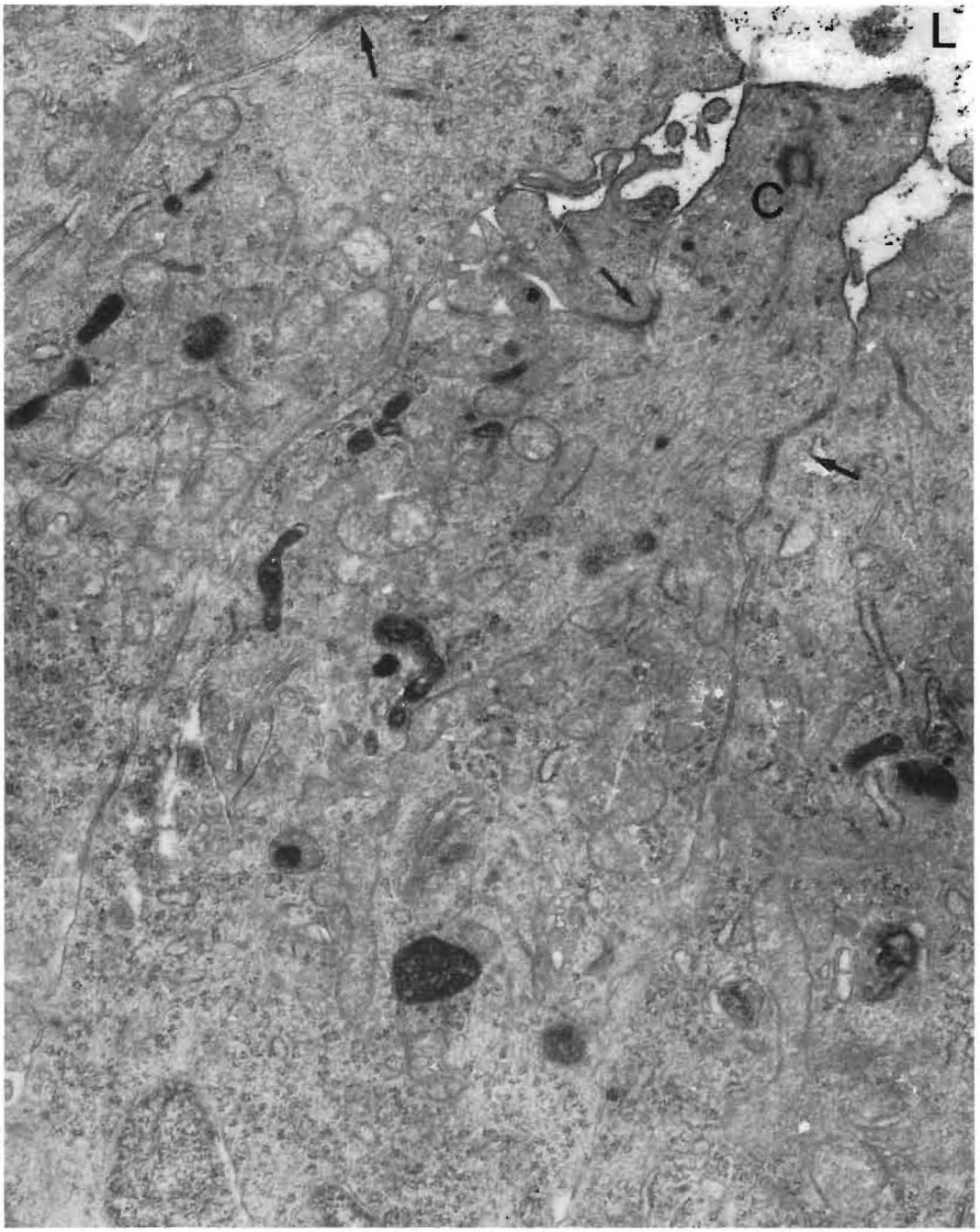

Fig. 20. 19-day-old embryo. Apical cytoplasm of a B pinealocyte limiting the follicular lumen (L). This zone is rich in organelles, microtubules and dense bodies of various morphology. At the level of the neck

The parenchyma adopts the form of ample follicles (fig. 16) and peripherally located vesicular formations.

The follicular walls demonstrate a clear predominance of the parafollicular zones. The follicular layer is ample and has a compact aspect. It is composed of tall cells, with their nuclei located in various layers in the lower half of the follicular zone. The apical cytoplasms are very well developed.

The B pinealocyte is the most abundant cell type of the parafollicular zone (fig. 17). Ultrastructurally it is a very differentiated cell in which a series of parts, similar to those described by *Collin* [1966a, b] in the magpie, can be distinguished. The *soma* contains its nucleus surrounded by a thin cytoplasmic lamina. It is an ample nucleus with lax chromatin and one or more manifest nucleoli. The nuclear membrane presents profound invaginations located in the superior pole of the nucleus, oriented toward the follicular lumen. A thin *basal prolongation* with a tortuous trajectory leaves the soma and heads toward the parafollicular zone. This prolongation contains clear vesicles and very characteristic dense granules. In the small follicles, the basal prolongations of the B pinealocytes and in the vicinity of the basal membrane.

The *apical cytoplasm* shows a regular stratification of its organelles. In the vicinity of the nucleus there is an accumulation of rough endoplasmic reticulum, in the form of a nuclear cape. Almost all of the B pinealocytes contain lipid inclusions, generally only one, of large size and laminar aspect, located in the immediate vicinity of the superior pole of the nucleus (fig. 19). We never found small lipid drops of a homogeneous structure in the B pinealocyte. In a more superior position, a well-developed Golgi system is located. In the highest portion of the supranuclear cytoplasm, abundant mitochondria and numerous microtubules are seen, which are very abundant in the vicinity of the neck. The abundant, large, dense, polymorphic bodies tend to accumulate in the superior part of the supranuclear cytoplasm (fig. 17–20).

The neck (fig. 21) corresponds to the narrowing of the apical cytoplasm in the vicinity of the lumen where the mechanisms of union are located. The neck contains numerous microtubules which join together from the apical cytoplasm. We now see in the follicular lumen a spherical dilation or *terminal drumstick*. The inferior part of the drumstick contains microtubules which diverge into a fan from the neck. Also, in the superior half there are mitochondria and ribosomes. One characteristic component of the terminal drumsticks are their ciliar prolongations (fig. 22). The basal corpuscles, in the form of diplosomes and frequently with ciliar roots, are located in these drumsticks. The cilia, as the transversal cuts demonstrate, lack the central pair of microtubules.

The rest of the follicular pinealocytes or A pinealocytes have nuclei that tend to be located in a deep position in the follicular zone. In this manner, two layers of nuclei can be distinguished: one superior with the nuclei of the B pinealocytes and the other inferior with the nuclei of the A pinealocytes. The nuclei of the A pinealocytes have angular forms and a dense chromatin in thick clusters which are very contrasted on a clear nucleoplasm. No profound invaginations are found in the nuclear membrane. The soma can be prolongated toward the parafollicular zone by means of a

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the mechanisms of union are characteristic between the cells (arrows), and a basal corpuscle can be seen in the terminal drumstick (C). × 10,000.

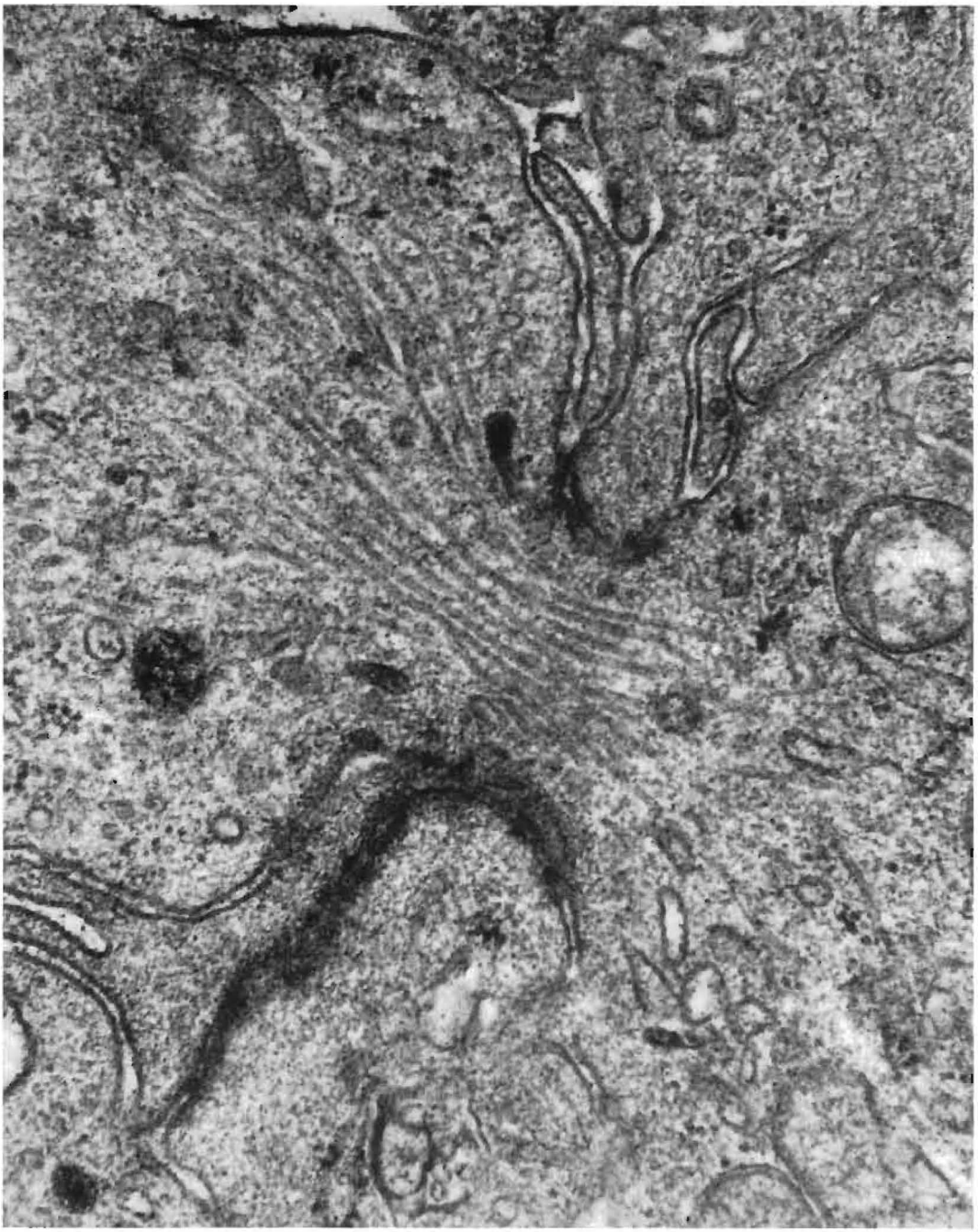

Fig. 21. 21-day-old embryo. Neck and part of the terminal drumstick of a B pinealocyte. Observe the existence of union mechanisms at this level and the richness of microtubules, which pass from the apical cytoplasm to the terminal drumstick by way of the neck. × 30,000.

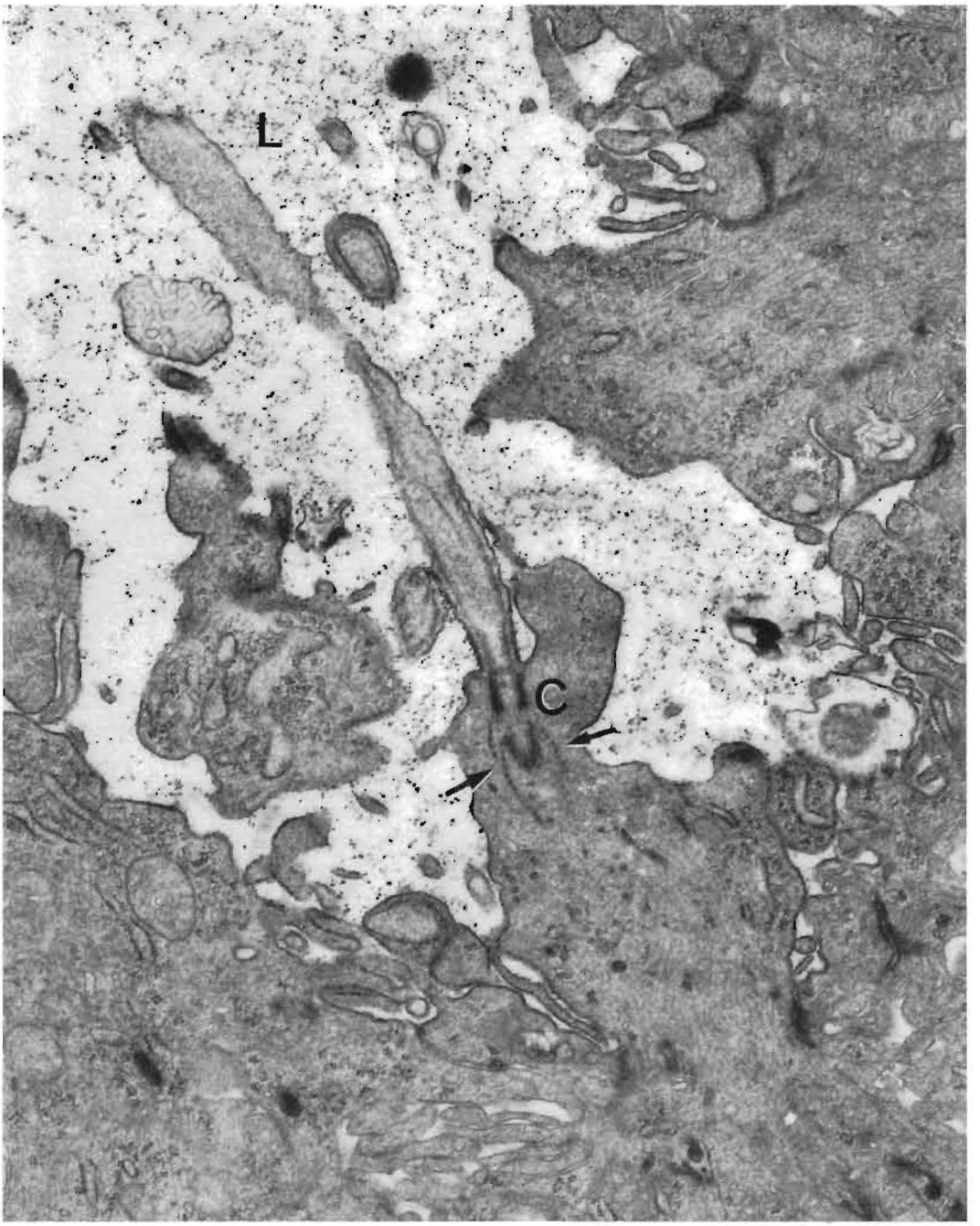

Fig. 22. 21-day-old embryo. Terminal drumstick of B pinealocytes in the follicular lumen (L). The existence of ciliar prolongations with a basal corpuscle (C) and small ciliar roots (arrows) is frequent in these drumsticks. × 10,000.

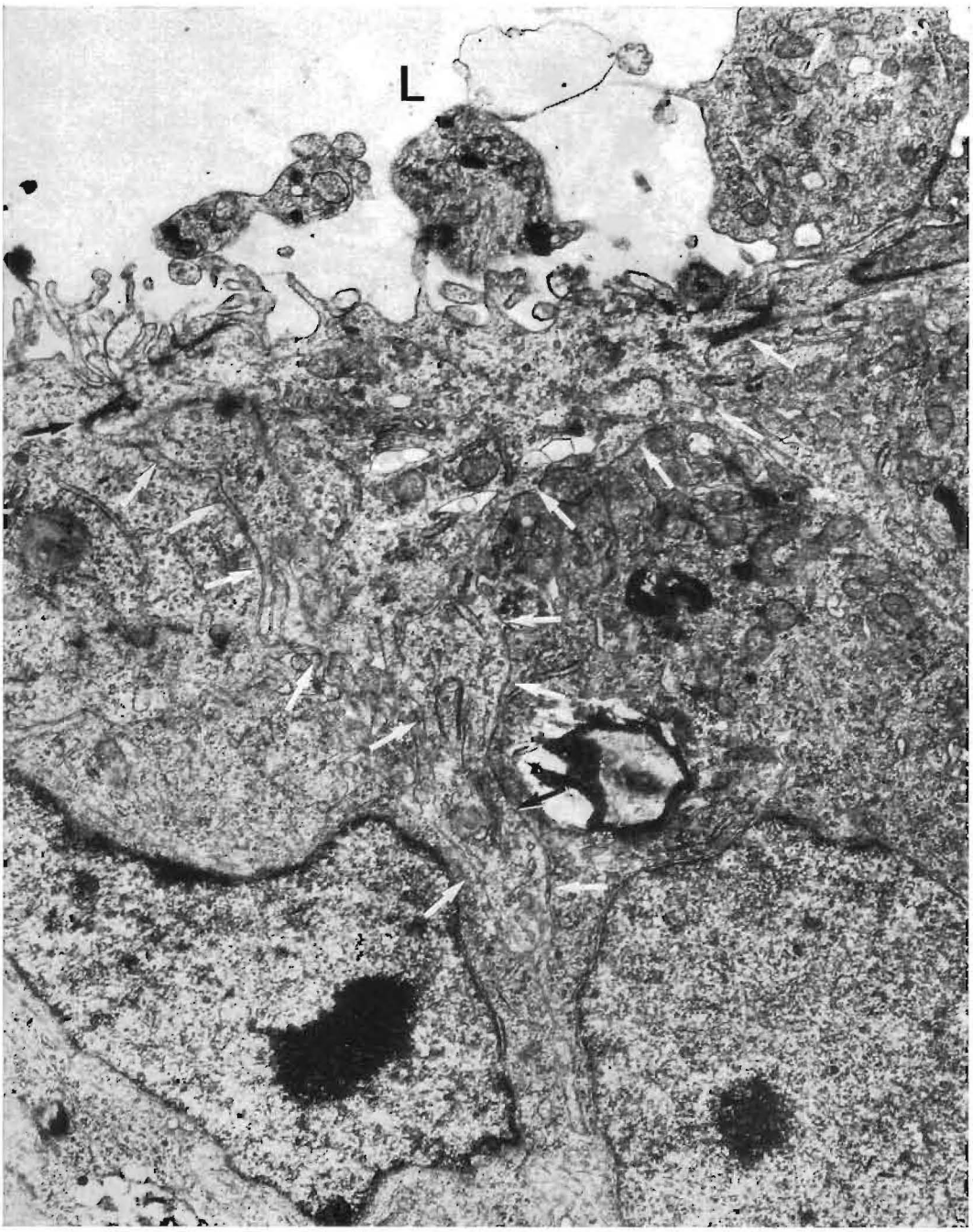

Fig. 23. 20-day-old embryo. Apical portion of an A pinealocyte widening to limit the follicular lumen (L). ×7,000.

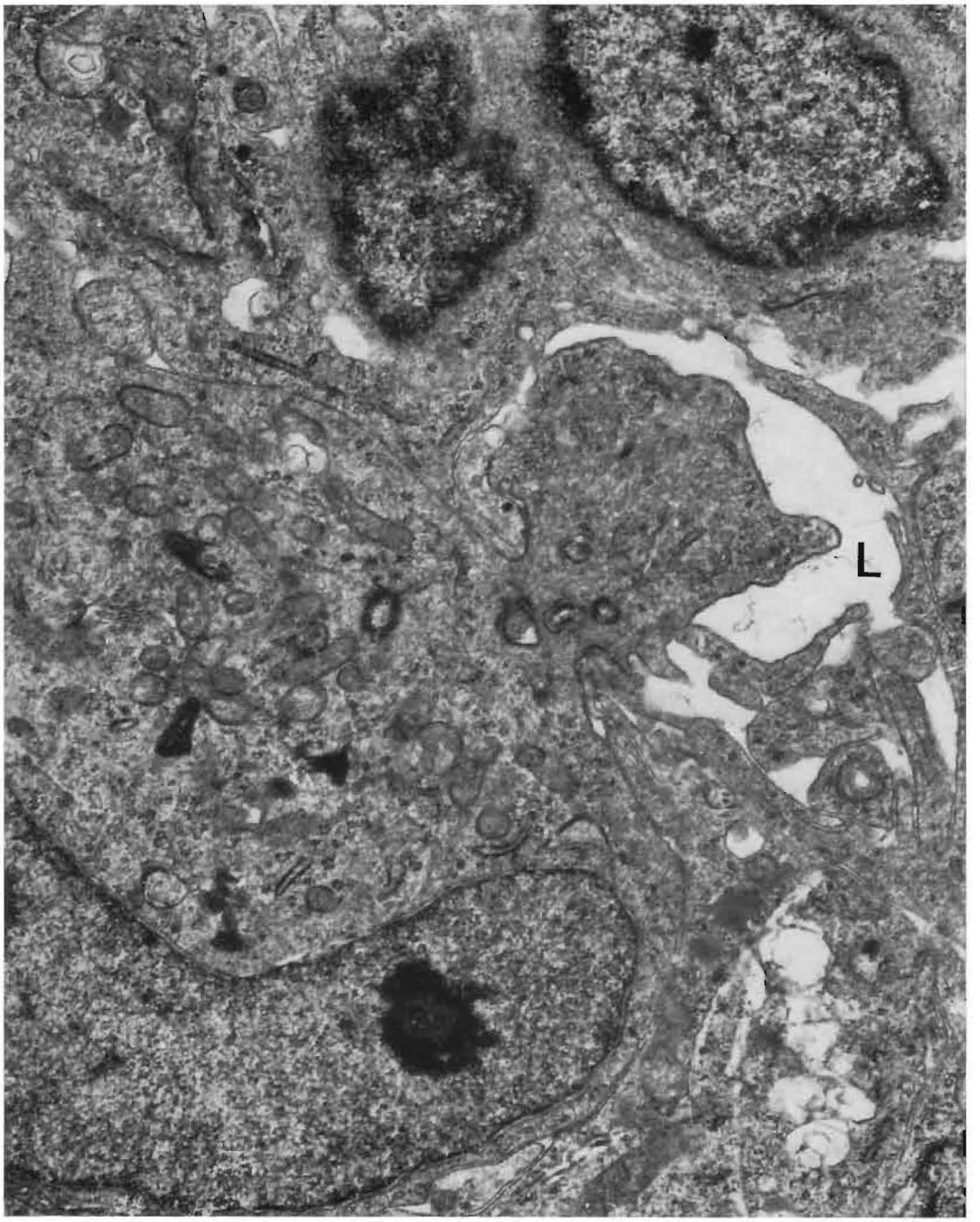

Fig. 24. 19-day-old embryo. Parafollicular zone. Formation of a cavity. Pinealocyte with terminal drumstick in the small lumen (L), in which the neck can already be distinguished. × 10,000.

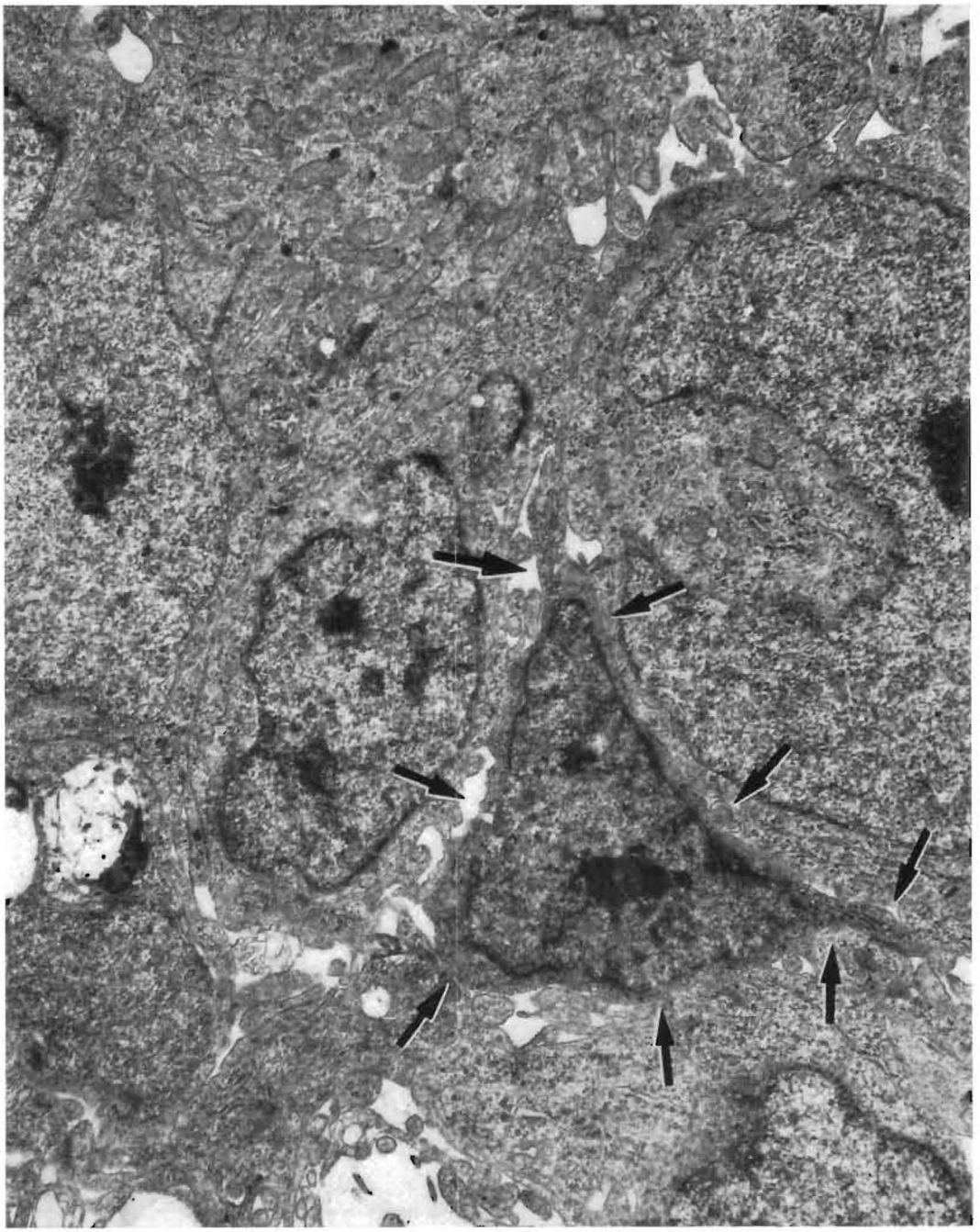

Fig. 25. 20-day-old embryo. Parafollicular zone.
A pinealocyte. × 7,000.

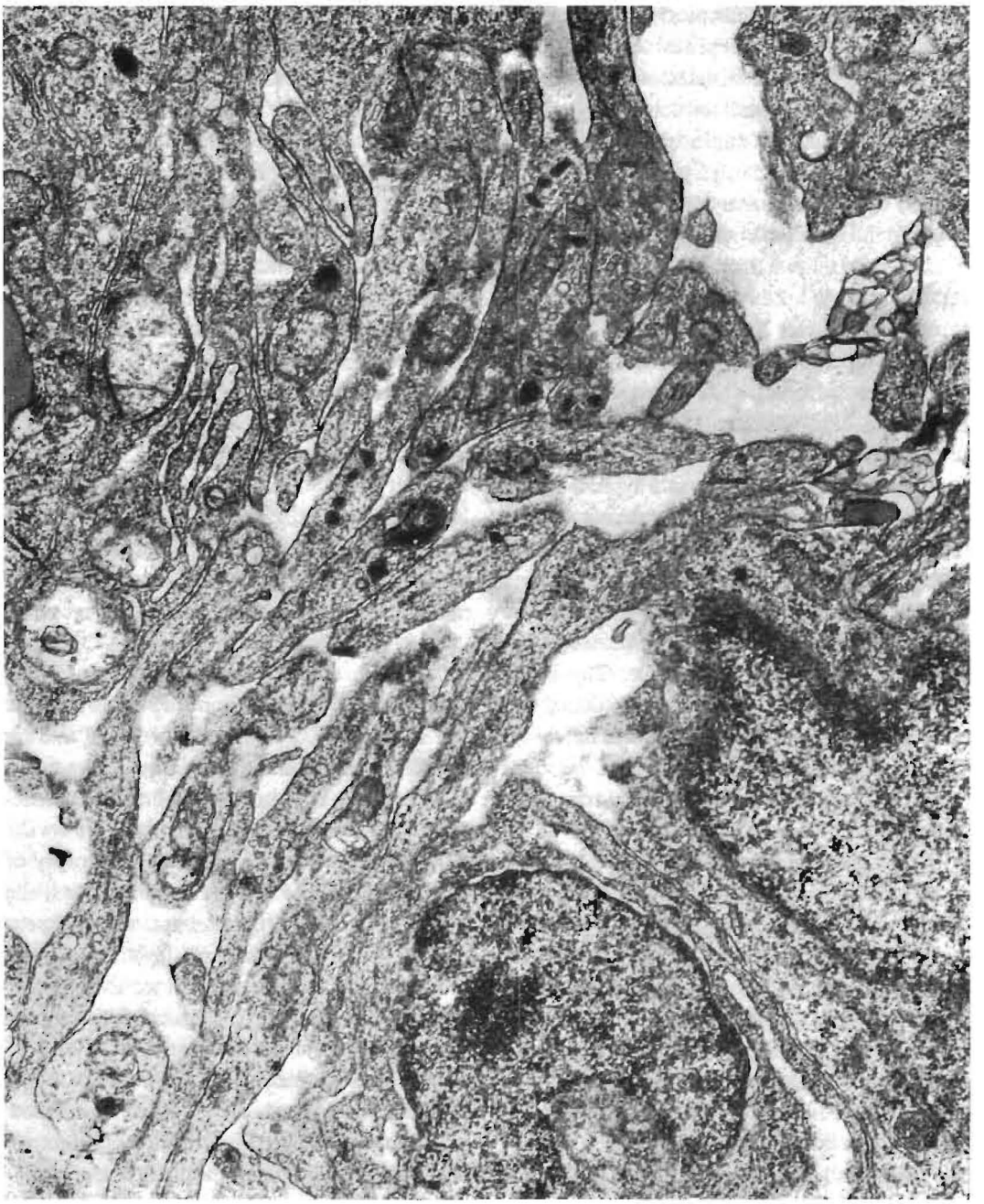

Fig. 26. 20-day-old embryo. Parafollicular zone. A group of prolongations from pinealocytes in the vicinity of the basal membrane (upper right-hand corner) with dense grains and clear vesicles.

basal prolongation. The apical cytoplasm, less developed than in the B pinealocyte, contains few ribosomes, short vesicles of rough endoplasmic reticulum, small mitochondria, microfilaments and some microtubules. All these structures are located in a hyaloplasm which is slightly electron-dense. One characteristic component are the abundant, small, round, homogeneous lipid drops. In no case did we encounter large lipid inclusions with a laminar aspect, as this was the case in the B pinealocytes. In the area around the follicular lumen we frequently found a dilation of the cytoplasm (fig. 23). The luminal terminal is flat with short, irregular, abundant microvillosities and, in some cases, it has a typical cilium (kinetocilia). There are also numerous covered vesicles in the portions of the cytoplasm proximal to the lumen. The A pinealocyte never forms drumsticks.

At 18–21 days of embryonic development the parafollicular zone is ample. The intercellular spaces have been notably reduced but can still be seen in the phases prior to hatching. The parafollicular zone is formed by cells joined in groups separated by cellular prolongations.

The parafollicular pinealocytes present the same morphologic types that have been described in the follicular zone, although the structural polarization is much less similar. The type B pinealocytes (fig. 24) tend to adopt globular forms: the nucleus is located eccentrically many times. In the other pole, the cytoplasm is very similar to the apical cytoplasm described in the follicular zone. The A pinealocytes have a less developed cytoplasm and tend to adopt a star shape (fig. 25).

In the older embryos, the parafollicular zone has thin prolongations, joined in groups, which head toward the basal membrane of the follicle, forming a fibrillar layer in the vicinity of the basal membrane. A large number of these prolongations contain clear vesicles and dense grains, and occasionally these are very abundant (fig. 26). These correspond to the prolongations of the B pinealocytes. The rest which present microfilaments and, frequently, lipid drops are those corresponding to the A pinealocytes.

In the parafollicular zone we also find numerous examples of formational cavities and some dense structures identifiable as degenerated cells.

Discussion

In the very earliest stages, the epithelium that forms the primary pineal outline is ultrastructurally similar to the neuroepithelium that lines the cavities of the central nervous system, as is demonstrated by some descriptions [*Fujita and Fujita,* 1963; *Strong,* 1964; *Tennyson,* 1964, 1970; *Smith,* 1966; *Mauryana and D'Agostino,* 1967; *Schluter,* 1973] and by our observations in the nervous tissue near the pineal. There are, however, some ultrastructural characteristics which are proper to the pineal outline, such as the greater quantity of the cytoplasm, the lack of prolongations, the existence of lipid drops, greater development of luminal projections and fewer free ribosomes – characteristics that we find in the neuroepithelium. In consequence, the cells of the pineal outline, even from the first stages of development, can be called 'pinealoblasts'.

The degenerated cells, which until now have not been described with the electron microscope, are a characteristic of the developing pineal. In one of our earlier studies [*Calvo and Boya,* 1977] with the optical microscope, we described, at 5–6 days, the existence of acidophilic bodies between the cells of the

pineal outline. These are present during the entire embryonic development but they predominate in the first phases. Various studies insist on the importance of the cellular degeneration in the morphogenesis of the nervous system [*Glucksmann,* 1951; *Kallen,* 1955; *Bodian,* 1966; *Cerro and Snider,* 1972; *Schluter,* 1973; *Schweichel and Merker,* 1973]. The ultrastructural aspect of the degenerated cells in the neuroepithelium is practically identical to those which we found in the pineal outline.

Until 9 days of embryonic development, the pineal grows by the formation of vesicles on the wall of the pineal recess. After the first 9 or 10 days, two zones differentiate in the wall of the vesicles which we call 'follicular' and 'parafollicular', following the terminology of *Boya and Zamorano* [1975]. The parafollicular layer corresponds to the hypendimocytes described by *Funkquist* [1912] with the optical microscope and accepted by the majority of earlier investigators, notwithstanding that only *Boya and Zamorano* [1975] have recognized this zone with the electron microscope in chickens of 2–5 days. The parafollicular zone appears in 9- to 10-day-old embryos and plays an important role in the pineal morphogenesis. Until the ultimate moments of development, the intercellular spaces visible in the pineal are localized only in this parafollicular zone; the follicular zone is always very compact. We believe that one of the missions of the parafollicular zone is to permit growth of the parenchyma, as in the spatial reorganization of the cells and their prolongations. The increase of pineal tissue does not consist in a simple cellular proliferation but these cells tend to organize, polarizing around a central lumen. This reorganization is possible due to the laxity of the parafollicular layer. The morphological translation of the reorganization of the pinealocytes are the formational cavities, visible in the parafollicular zone after 12 days of incubation. There is not any description of these structures in any literature. Especially in the first phases, the formational cavities can be interpreted as simple mechanisms of union. Notwithstanding, we always encountered, even in the earliest phases, characteristics which are normally associated with periluminal cytoplasm, such as radial organization, lipid drops, cilia, mitosis, etc. Never did we find mechanisms of union in zones without these characteristics. The advanced formational cavities can be interpreted as tangential cuts of a follicular lumen. Also, from a different ultrastructural aspect of the apical cytoplasm of the follicular pinealocytes, we found numerous examples of formational cavities very close to the basal membrane or in follicles cut through their midplane. In both cases, the only explanation is to admit an enormous irregularity of the follicular lumen. Complete observations, whether with the optical microscope or with the electron microscope, demonstrate follicular lumens of a spherical or ovoid contour surrounded by a very thick cell wall. The cellular rosettes described with the optical microscope by ourselves [*Calvo and Boya,* 1977] correspond to formational cavities, probably in very early phases.

After 9–10 days of incubation, the differentiation of the B pinealocyte, which will convert into the principal cell type in the pineal of the chicken, begins. In the following days, the B pinealocytes progressively increase in proportion, at that time developing the ultrastructural characteristics typical of this cell type. In 1974, *Wainwright* studied the evolution of HIOMT and 5-hydroxytryptophan decarboxylase during the pineal development of the chicken. Both enzymes are detected, for

the first time, at 10–12 days and increase progressively and rapidly at the moment of hatching. There is a clear parallel with the morphological and structural evolution of the B pinealocyte.

In the more mature embryos the B pinealocytes have an aspect similar to those described in other species of birds after hatching [*Collin,* 1966a, b, 1967, 1968, 1969, 1971; *Oksche and Vaupel von Harnack,* 1966; *Fujie,* 1968; *Bischoff,* 1969; *Hodges,* 1974; *Boya and Zamorano,* 1975; *Collin et al.,* 1975]. There are, however, ultrastructural characteristics that, until now, have not been described, such as (a) invaginations of the nuclear envelope, located in the superior pole of the nucleus; (b) polymorphic dense bodies which surely correspond to the I_1 inclusions of *Collin* [1966a, b, 1967, 1969, 1971] described in the magpie. This author considered them to be lysomes. Our morphological observations support this idea also, although we consider them to be typical of the B pinealocytes. We remember that *Beattie and Glenny* [1966] found an intense acid phosphatase activity in the apical cytoplasm of the pinealocytes; (c) laminar lipids whose ultrastructure and location are totally characteristic of this cellular type. These structures have been described, after hatching, only by *Boya and Zamorano* [1975]. The laminar aspect is probably due to a phenomenon of extraction during the fixation or inclusion of the samples. However, this reflects a chemical difference with respect to the lipids of the A pinealocytes, because these lipids are never affected by the extraction phenomenon.

The second pineal cell type, the A pinealocyte, has a simplier structure similar to the 'pinealoblasts'. Only in the last third of development, this cell shows ultrastructural characteristics which permit an easy distinction between them and the 'pinealoblasts' of the early phases of embryonic development. The morphological data of the A pinealocytes speak in favor of a supportive role for these cells, which is accepted by most authors.

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J. Calvo, Department of Histology and General Embryology, Faculty of Medicine, University Complutense, Madrid (Spain)