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Evolution of the pineal gland in the adult chicken

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Abstract. The structural pattern of the pineal gland in the hen corresponds to a more advanced stage of the evolution which began in an early period of the animal's life. This evolution corresponds mainly to the transformation of the large follicular cavities into cellular 'rosettes'. The parafollicular layer disappears from the rosette wall which thus remains with only one row of cells (A and B pinealocytes).

The cellular hypertrophy and the great development of the pinealocyte organelles in the adult pineal gland makes us think of this gland as a functionally active organ. This functional activity must have remained during the entire period of the time studied (1–5 years), due to the ultrastructural uniformity found and due to the fact that we could not observe any type of degenerative process in the gland.

Introduction

Morphological research on the bird pineal gland has been centred basically on the identification of photoreceptor structures. These structures possess a very rudimentary character in these species [*Collin,* 1966a, b, 1967, 1968, 1969, 1971; *Oksche and Vaupel von Harnack,* 1966; *Bischoff,* 1969; *Oksche and Kirschtein,* 1969; *Oksche et al.,* 1972; *Collin et al.,* 1976]. In many of these species, however, there are no detailed descriptions of the histological structure of the pineal gland.

In a series of previous papers we have described the structure of the pineal gland during its embryonic development [*Calvo and Boya,* 1977a–d] and its post-hatching morphological evolution [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977a, b]. We have as well described certain particular aspects of it, such as the innervation development [*Calvo and Boya,* 1977a–d] and the lysosomal nature of the polymorphous dense bodies in the B pinealocytes [*Calvo and Boya,* 1977a–d]. We believe that the knowledge of the normal structural pattern of the pineal gland is a previous and necessary requirement in order to interpret the possible morphological variations provoked by the different experimental situations. Thus, pineal morphology may be an important research tool for the knowledge of pineal function.

In this paper, we study the pineal structure during adult life from a light-microscopical, histochemical and ultrastructural point of view.

Materials and methods

Hen pineals of 1, 2, 3, 4, and 5 years of age were used for our study. The hens were always maintained under natural conditions of light and feeding. The animals were sacrifized by decapitation and the pineal glands were fixed immediately by immersion in the corresponding fixative.

The fixatives used for light-microscopical studies were 10% formalin and Bouin's fluid. The samples were embedded in paraffin and serially sectioned at 7 μm of width. The staining techniques used were HE, Van Gieson, PAS and the Gomori technique for reticular fibres.

The histochemical studies were donc in pineals fixed in 0.1 *M* cacodylate-buffered 3% glutaraldehyde, pH 7.4, during 2 h at 4 °C. After washing the samples in cacodylate buffer, the sections were obtained from a freezing microtome. The sections were incubated 30–45 min at 37 °C in *Miller and Palade*'s [1964] modification of the Gomori medium for acid phosphatase. Part of the sections were reduced in ammonium sulphite for study with the light microscope. The unreduced sections were post-fixed and embedded in Vestopal W for study with the electron microscope.

For the ultrastructural studies, the pineals were fixed in 0.1 *M* phosphate-buffered 5% glutaraldehyde, pH 7.4. The post-fixation was done with phosphate-buffered 2% osmium tetroxide. After dehydration in acetone, the samples were embedded in Vestopal W. The sections were stained with uranyl acetate and lead citrate and then studied in an EM 201 Philips microscope.

Results

Light microscopy

The pineal parenchyma of the adult hen presents a compact aspect under the light microscope, and there are no large variations along the age intervals studied. The follicular cavities, typical in young animals, are not found here (fig. 1). Also there is not clear limit between the follicular and parafollicular layers as found in previous ages (fig. 1). Instead of all this, the parenchymal cells show an apparently homogeneous arrangement. This gives the parenchyma uniform appearance when observed at low magnification. A detailed study, however, proves clearly that the pinealocytes group themselves in radial arrangements forming cellular rosettes. The entire pineal parenchyma is composed of these rosettes. Each rosette contains only one row of nuclei arranged in a circular fashion. Inside the row of the nuclei, perfectly lined, there is a band with no nuclei corresponding to the supranuclear portion of the cytoplasm of the radially arranged pinealocytes. We could not find lumens in the centre of the rosettes. Occasionally, we found formations of larger diameter that frequently do not arrange themselves in a circular fashion but rather in a more irregular way. Also, their supranuclear cytoplasms are broader. The centre of these formations may present an empty cavity of small diameter and irregular borders. Sometimes, the centre is totally occupied by the apical cytoplasms of the pinealocytes or by a small group of cells.

The modification of *Miller and Palade* [1964] of the Gomori technique for acid phosphatase shows the existence of a great amount of lysosomes in the pineal parenchyma of the adult hen (fig. 2). All the pineal lysosomes place themselves in circular arrangements or lysosomal 'rosettes' whose size and distribution are parallel to the cellular rosettes observed with aniline techniques.

The stroma of the adult pineal gland is more dense and fibrous than that of young animals. The distribution pattern of the pineal stroma shows variations in the course of adult life. This evolution, already indicated in previous studies [*Boya and Calvo,* 1977a, b; *Calvo and Boya,* 1977a–d], consists of a progressive fragmentation of the pineal parenchyma due to the penetration of thin connective sheets. In the pineals of the oldest animals studied (5

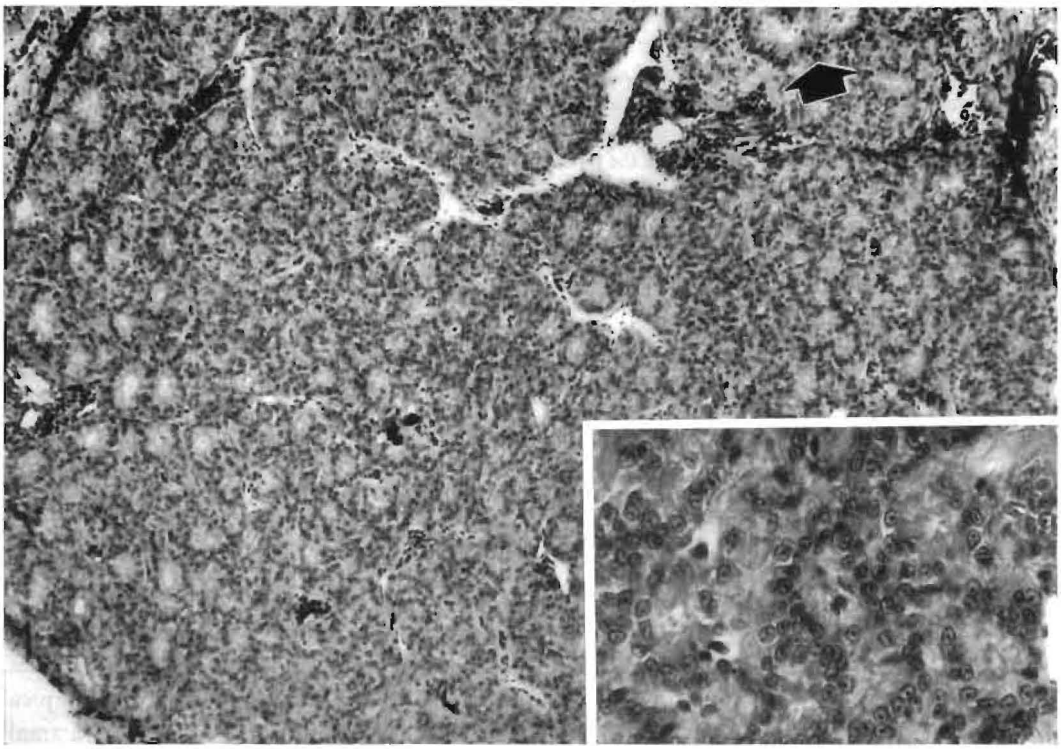


Fig. 1. 4-year-old hen. Compact aspect of the pineal parenchyma formed by many small pinealocyte rosettes. There are also a few larger formations of a more irregular arrangement (arrow). HE.

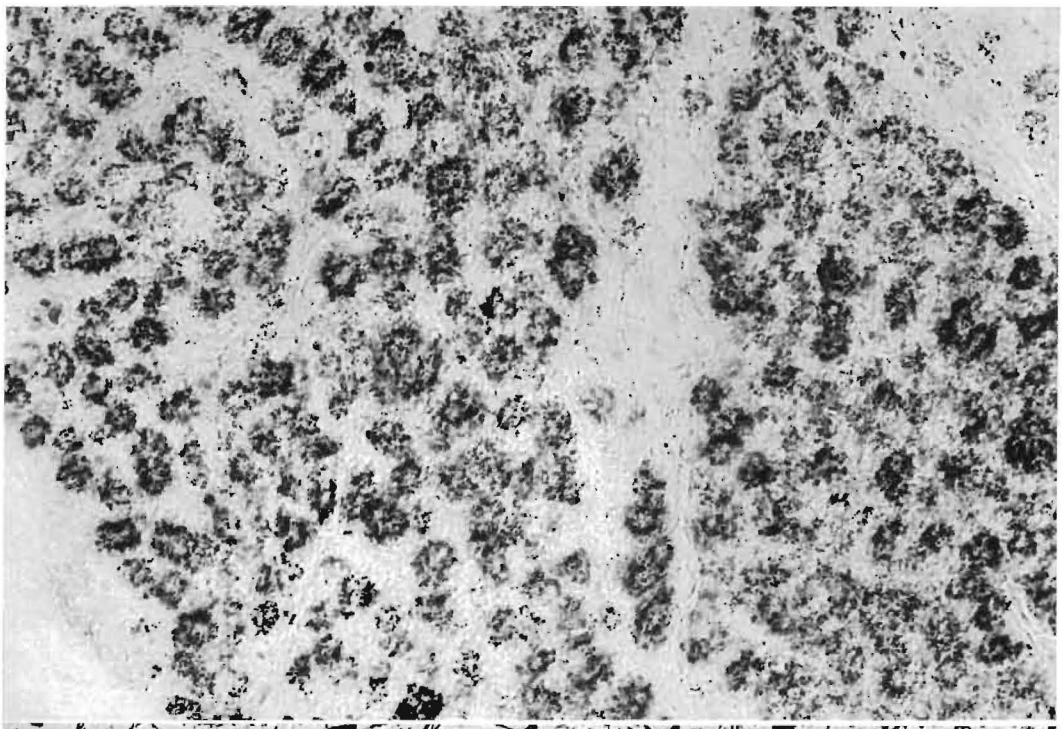
years old), the parenchyma appears divided in small territories by thin connective septa (fig. 3). Most of these territories contain only one cellular rosette. The cellular component of these vascular-connective septa presents no variations with respect to the descriptions of of previous phases. The presence of lymphoid tissue in the pineal stroma of the adult phase is worth to be pointed out.

Electron microscopy

The pineal parenchyma of the adult hen presents a solid aspect, using the low magnification of the electron microscope, due to the absence of follicular cavities typical of young animals [*Boya and Calvo,* 1977a, b]. This aspect remains all along the age intervals studied. A more detailed study shows, however, the existence of a great amount of lumina bordered by a line of junctional mechanisms

Fig. 2. 3-year-old hen. Reaction to acid phosphatase. Note the abundance of lysosomes and their arrangement in rosettes. The larger cavities are more scarce.

Fig. 3. 5-year-old hen. Intense fragmentation of the pineal parenchyma in small territories limited by thin connective-vascular septa. Gomori.



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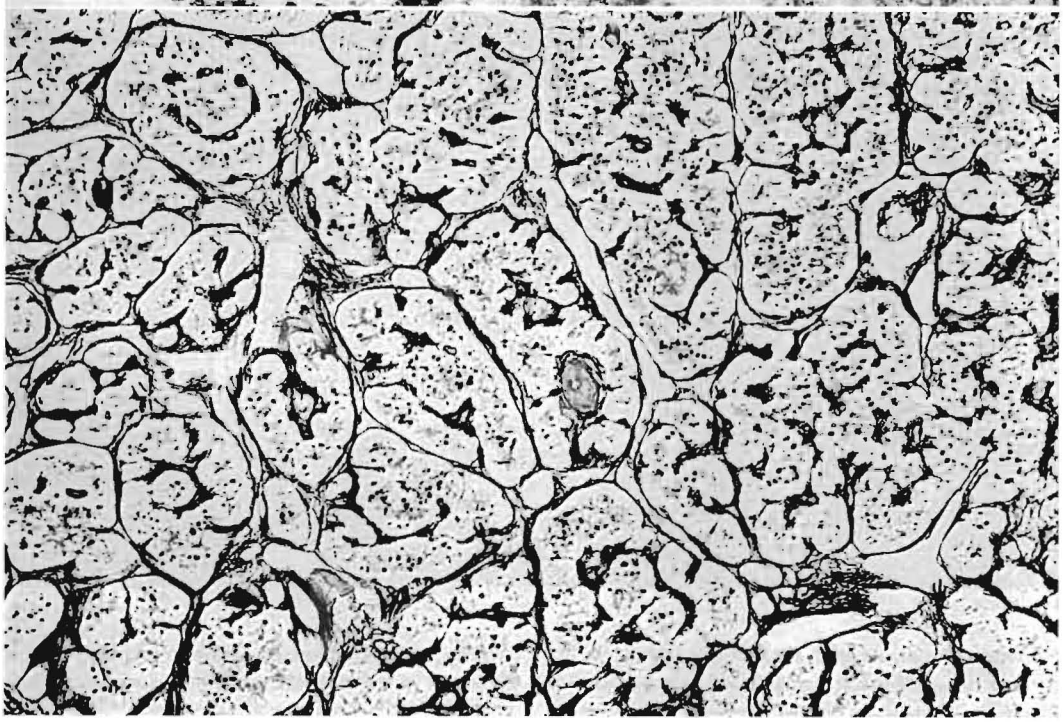

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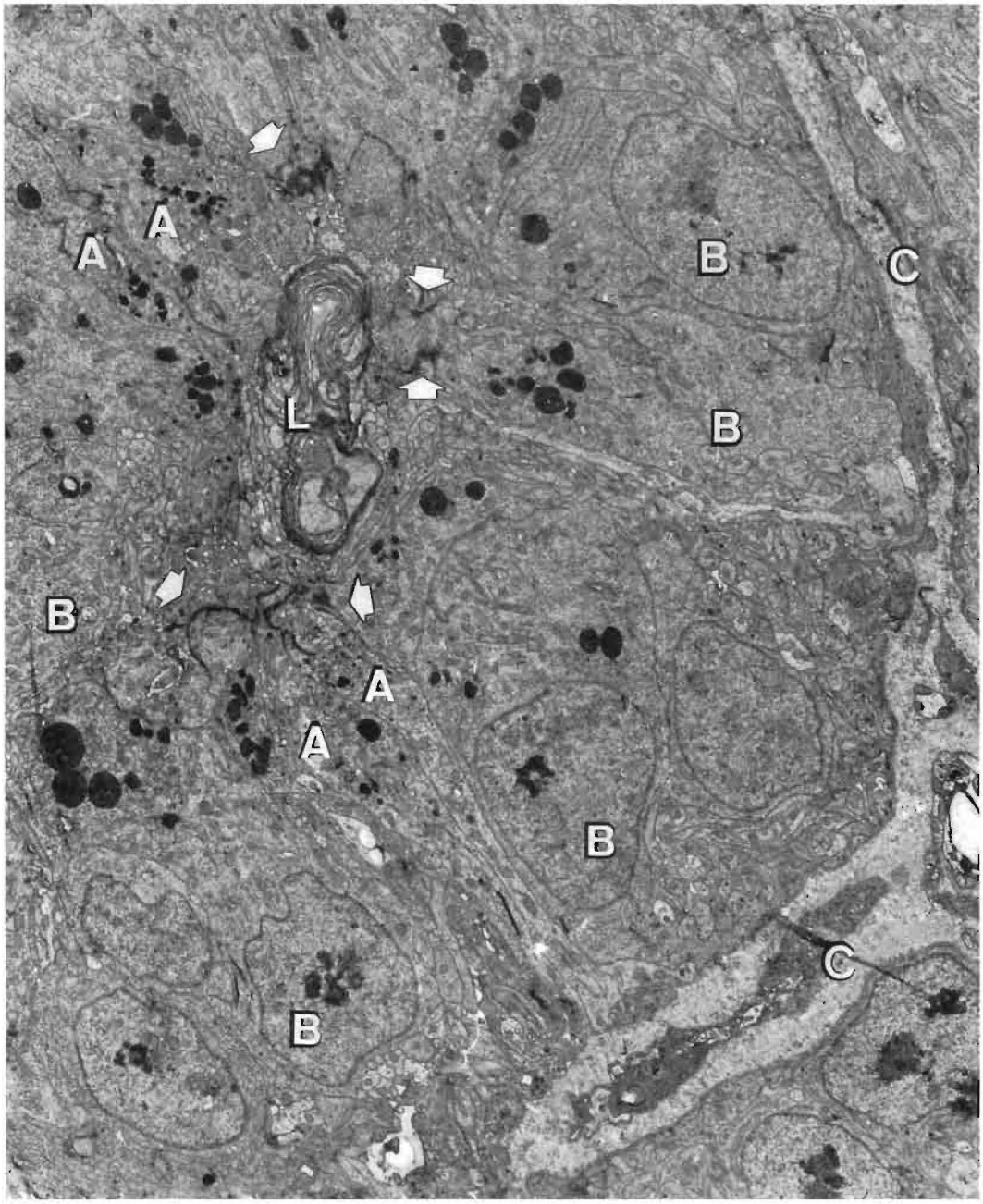


Fig. 4. 3-year-old hen. Wall of a small cavity (L) formed by a single layer of A (A) and B (B) pinealocytes. The cavity (L) is occupied by whorl-like lamellar systems, ciliary processes and terminal clubs. It is limited by a series of junctional mechanisms (arrows). C = Connective-vascular septa. × 3,000.

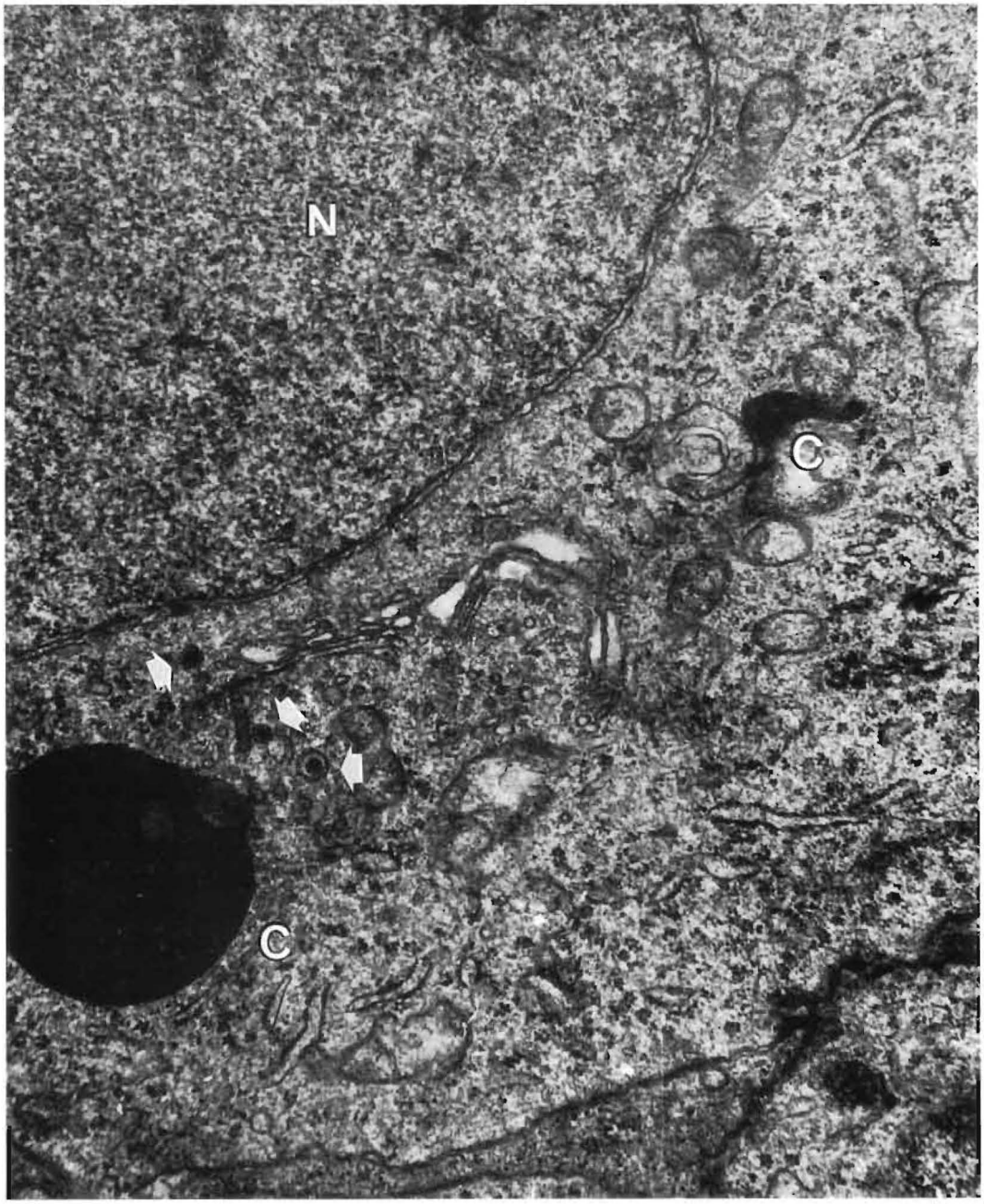


Fig. 5. 5-year-old hen. B pinealocyte. Golgi commex in the vicinity of the nucleus (N). Presence of plicrovesicles and larger dense-cored vesicles (arrows). Dense bodies (C). In the largest one, droplets of a lipid aspect can be observed. × 15,000.

(fig. 4). Although these lumina are totally occupied by the apical processes of the pinealocytes, they are easily identified due to the existence of a band of junctional mechanisms that border the lumen. This explains the solid aspect observed at low magnifications. The pinealocytes are placed in a radial arrangement with respect to the lumina. The pattern of radial arrangement is more regular as the diameter of the lumen becomes larger. There are also variations in the content of the lumina in relation to their size. The lumina of larger diameter usually present whorl-like lamellar structures which are sometimes very well developed (fig. 4, 10).

In the pineal of the adult hen there is, between the follicular and parafollicular layers, no parenchymal division as it is present in young animals (fig. 4) [*Boya and Calvo,* 1977a, b]. The parafollicular layer has disappeared. Therefore, due to the great number of lumina and the polarized aspect of the pinealocytes, we may admit that all the parenchymal cells are in contact with a central lumen (fig. 4).

The stroma of the pineal gland of the adult hen is very rich in collagen microfibrils. Excepting this larger fibrillar density, the connective tissue of the adult pineal presents the same components (blood vessels, connective cells, unmyelinated nerve fibres, and lymphoid cells) as described in previous phases [*Boya and Calvo,* 1977a, b; *Calvo and Boya,* 1977a–d]. In addition to the thick connective septa, there are many, sometimes very thin, sheets of stroma distributed all around the gland. These sheets tend to divide the pineal parenchyma in very small territories, which contain a small amount of lumina with their corresponding pinealocytes arranged radially. We frequently find pinealocytes whose nuclei (somas) are located in the vicinity of the basement membrane (fig. 4). In any case, the distance between the pinealocyte soma and the connective space is always very small in adult pineals.

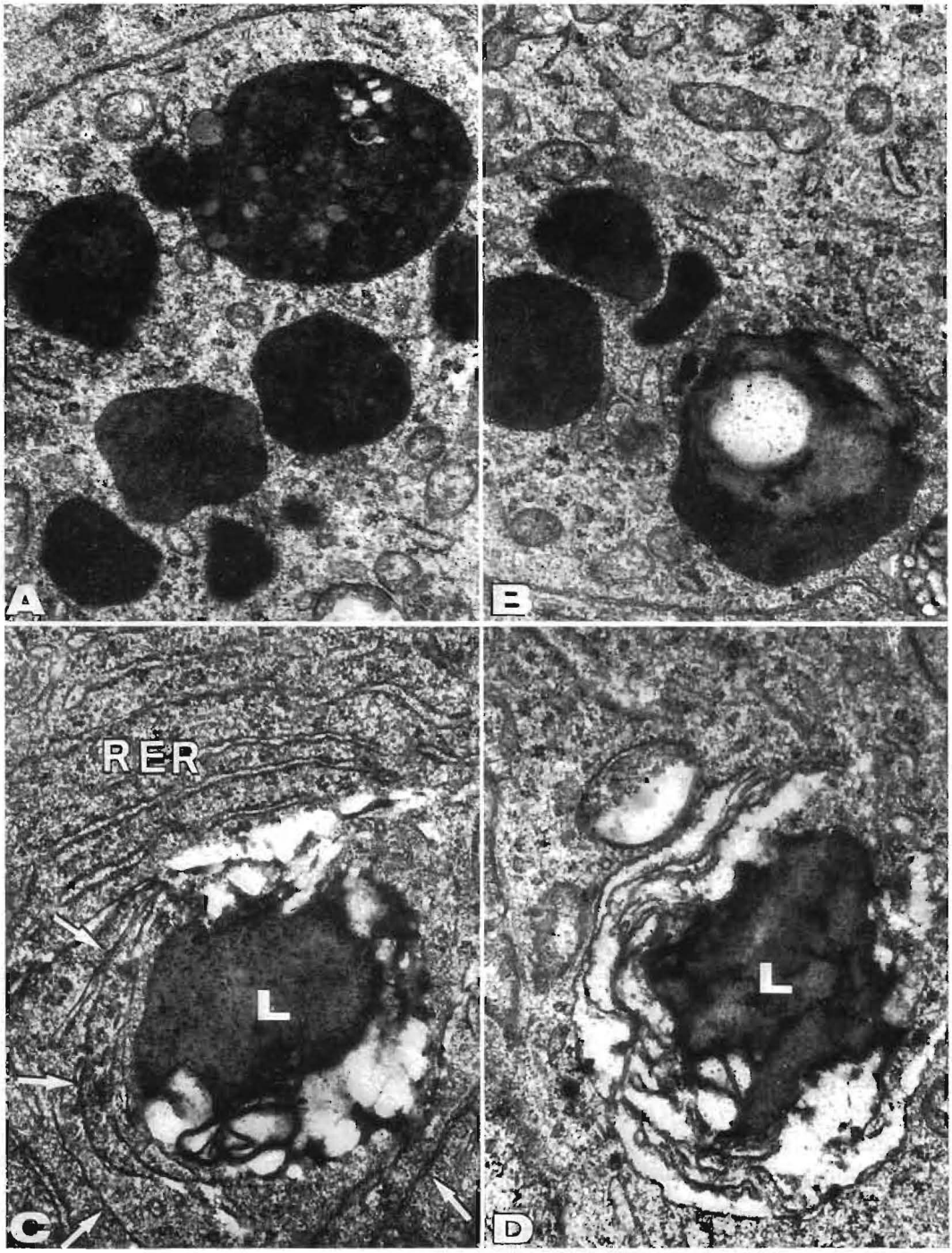
The pineal parenchyma of the adult hen presents both cellular types, A and B pinealocytes, which have been previously described in embryos and post-hatching stages [*Boya and Calvo,* 1977a, b; *Calvo and Boya,* 1977a–d].

The B pinealocyte is the most abundant cellular type (fig. 4). These cells present a marked hypertrophy which gives them a globular aspect characteristic of this adult age. The nucleus is large and has a round or ovoid shape. It presents dispersed chromatin and several very prominent nucleoli (fig. 4).

The cytoplasm of the B pinealocyte is very rich in organelles. The rough endoplasmic reticulum is located in the vicinity of the nucleus and in the periphery of the apical cytoplasm. It appears in the form of long cisterns that place themselves in a parallel fashion. All the cytoplasm is very rich in free polyribosomes (fig. 4).

The Golgi complex is very well developed. It is located immediately above the nucleus. Related to the Golgi complex we found numer-

Fig. 6. **A** 5-year-old hen. Large polymorphous dense bodies in a B pinealocyte. Note the existence of several lipid-like droplets in the largest one. × 15,000. **B** 5-year-old hen. Polymorphous dense bodies in the cytoplasm of a B pinealocyte. The largest one presents a single, large lipid-like droplet. × 20,000. **C** 4-year-old hen. B pinealocyte. Lamellar lipid formed by a lipid-like central material (L) and a peripheral lamellar system (arrows) which corresponds to cisterns without ribosomes. The space between this lamina is occupied by cytoplasmic bands. Note the development of the rough endoplasmic reticulum (RER) in the vicinity of the lamellar lipid and its relation to it. × 30,000. **D** 5-year-old hen. B pinealocyte. Lamellar lipid better limited than that in the 4-year-old hen (c). Note the unprecise limit of the lipid centre (L) and the peripheral membrane system free of ribosomes. × 20,000.


A
B
RER
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ous clear vesicles as well as coated vesicles. There are also dense-cored vesicles of a larger diameter (800–1,500 Å). We have also found formations of these vesicles in the Golgi complex (fig. 5).

The rest of the apical cytoplasm contains numerous mitochondria, which are more abundant than in previous phases (fig. 4). There are also microtubules in the entire cytoplasm of the B pinealocyte.

The 'polymorphous dense bodies' are the most characteristic components in the B pinealocytes of the hen pineal (fig. 4, 5, 10). They are located immediately above the Golgi complex interspersed among the mitochondria which are also located here. The polymorphous dense bodies of the adult pineals present round shapes. Only in those of smaller size do we find the irregular morphology which gives the name to those structures. They are considerably larger than those found in young animals. Most of them present a diameter near 1 μm, although it is not rare to find larger dense bodies whose diameter lies between 1.5 and 2 μm. Their content is homogeneous and very electron-dense. During adult life, however, many polymorphous dense bodies present several round droplets in their interior. These well-limited droplets are homogeneous and their content is less electron-dense than the rest of the body content (fig. 5, 6a). These droplets are more frequent in the larger-sized bodies; their size is approximately 0.2 μm. Their shape and electron density remind us very much of lipid droplets. Sometimes, these droplets seem to fuse in order to form one single droplet which is surrounded by all the rest of the electron-dense material (fig. 6a). We have never found isolated homogeneous lipid droplets in the cytoplasm of the B pinealocytes. These droplets have always been found in relation to the polymorphous dense bodies.

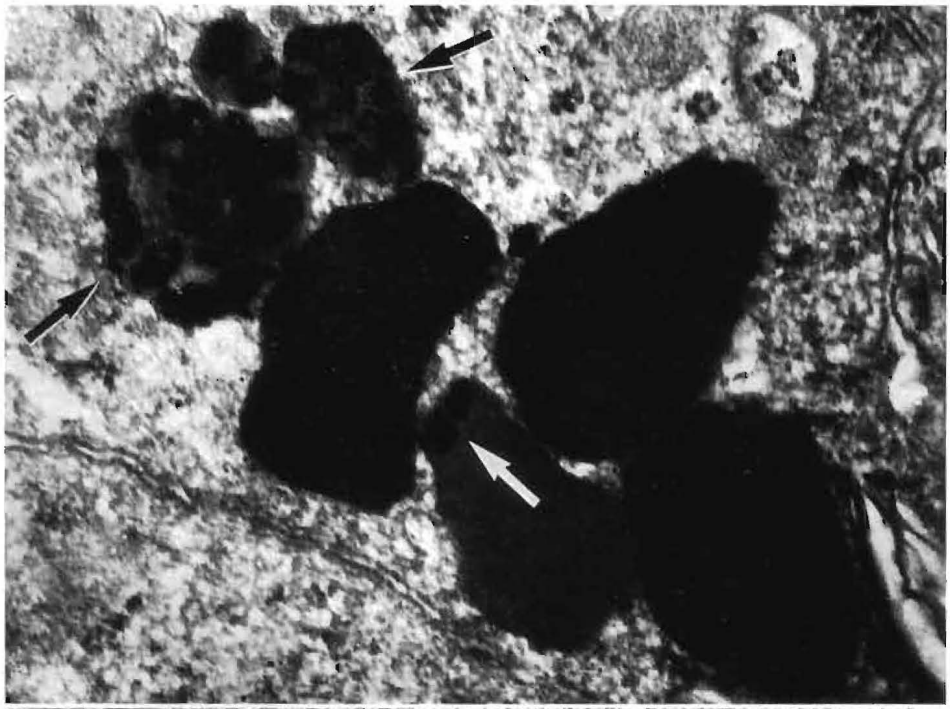
The ultrastructural study of sections incubated for acid phosphatase proves the lysosomal nature of the polymorphous dense bodies, as has been described in previous papers [*Calvo and Boya,* 1977a–d]. In the interior of these dense bodies we found a lead phosphate precipitate, perfectly visible even in contrasted sections (with uranyl acetate and lead citrate), which indicates the presence of acid phosphatase in them (fig. 7, 8). But the aspect of this precipitate varies greatly among them. Mostly it appears in a homogeneous fashion occupying the whole interior of the particle. Other times we found thick granules scattered irregularly in the interior of the dense body (fig. 7). Only in some cases, we found one or two granules. Occasionally, we have found dense bodies with no enzymatic reaction. When the dense bodies present lipid droplets, they lack lead phosphate precipitate.

The Golgi complex of some B pinealocytes presents reaction product for acid phosphatase. However, in most of the B pinealocytes the Golgi complex lacks acid phosphatase activity. No other component of the B pinealocyte, excluding the above mentioned, presents this type of activity.

Finally, in the cytoplasm of the B pinealocytes in the hen, we frequently find what we have named 'lamellar lipids' (fig. 6, 8) [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977a b;

Fig. 7. 3-year-old hen. B pinealocyte. Polymorphous dense bodies with a positive reaction to acid phosphatase. The lead phosphate precipitate appears in a homogeneous fashion in some dense bodies. In others it appears in the form of granules distributed irregularly in the interior of the dense body (arrows). × 30,000.

Fig. 8. 4-year-old hen. B pinealocyte. Polymorphous dense body with a positive reaction to acid phosphatase in relation to a lamellar lipid. × 20,000.



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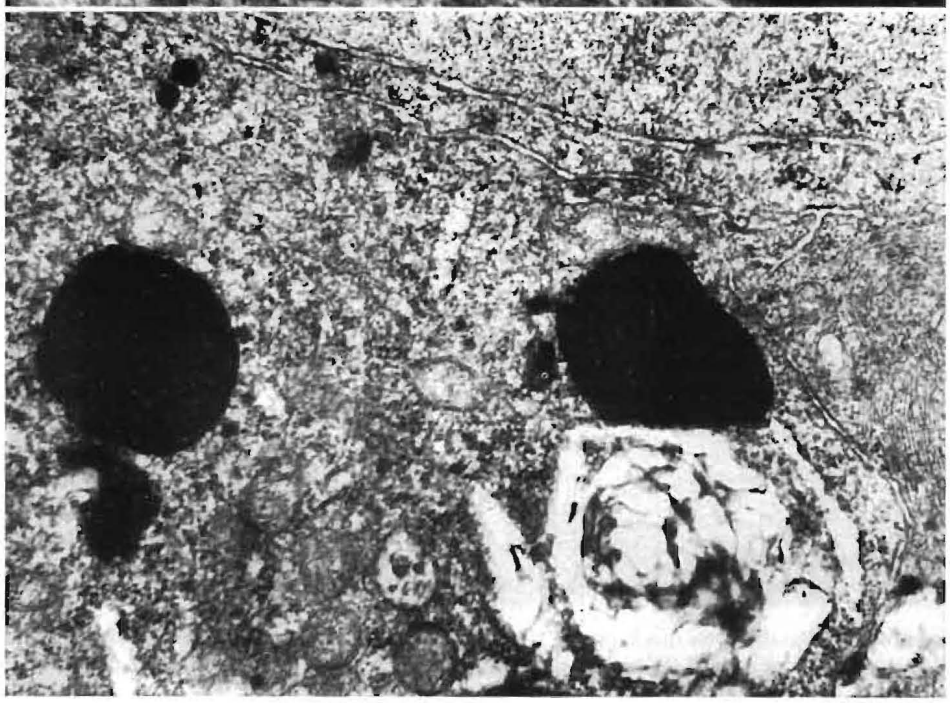

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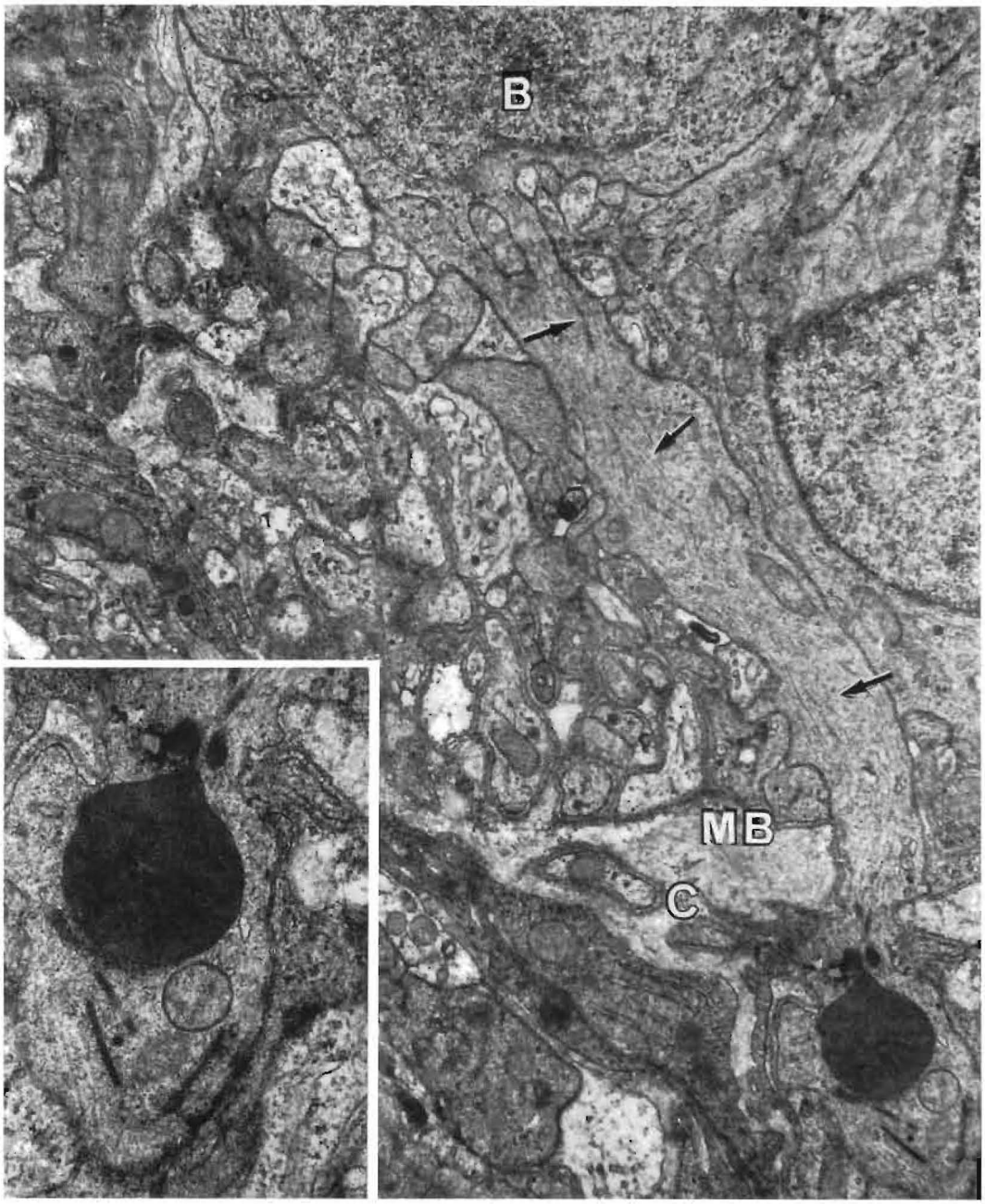


Fig. 9. 4-year-old hen. Basal process of a B pinealocyte (B) which ends in the vicinity of the basement membrane (MB) that limits the vascular-connective space (C). Note the abundance of microtubules (arrows) and the existence of dense bodies (one of them is very large) and bars near the end of the process. × 7,000. Inset: × 30,000.

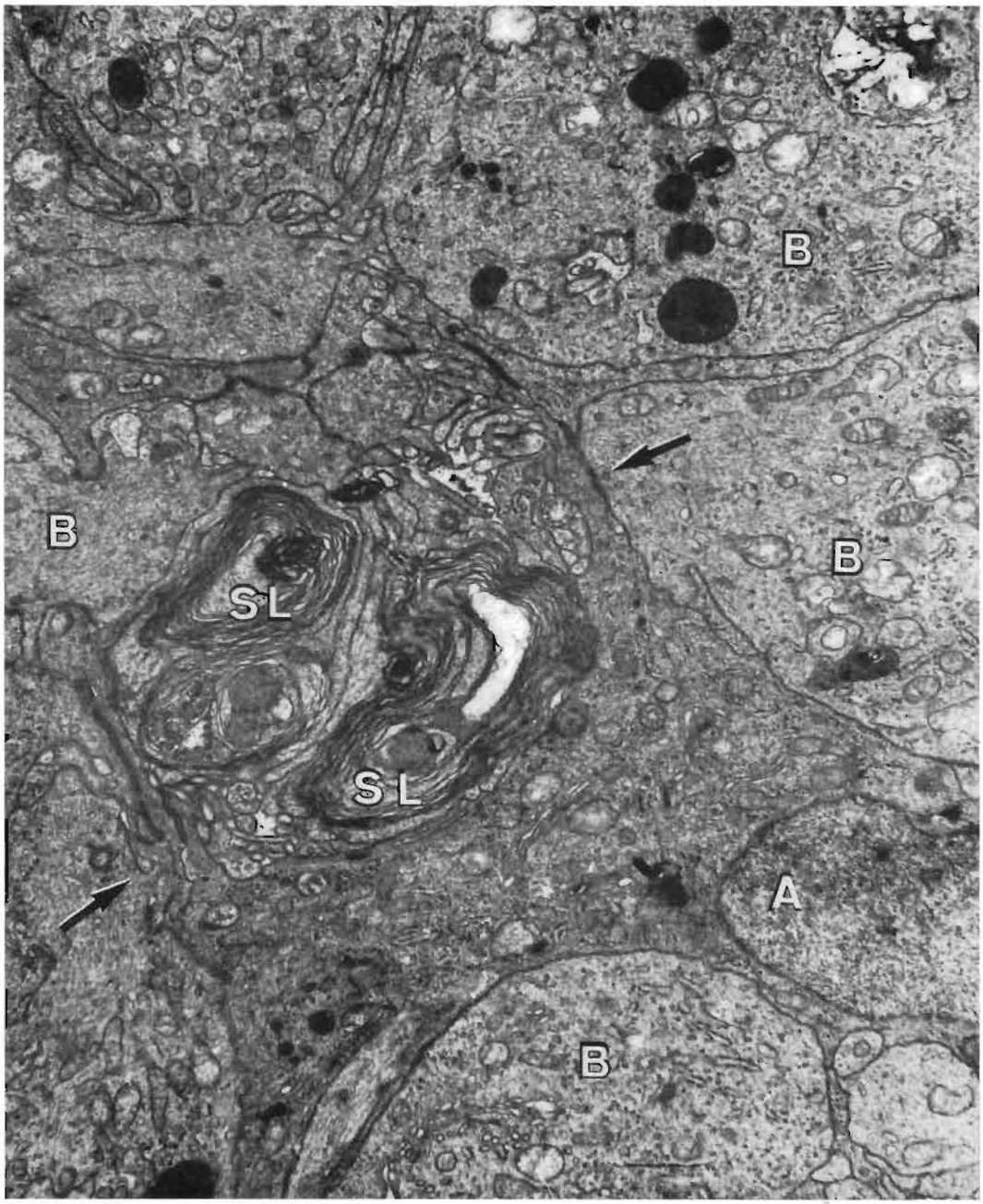


Fig. 10. 5-year-old hen. Small cavity occupied largely by whorl-like lamellar systems (SL). In its wall we may observe B pinealocytes (B) and the supranuclear portion of a dense cell type A pinealocyte which widens to border the cavity. It is related to the adjacent cytoplasms by junctional mechanisms (arrows). × 7,000.

Calvo and Boya, 1977a–d]. They are located in the immediate vicinity of the nucleus and, although they are generally single, there may be more than one per cell. The size is difficult to determine since they lack clean limits, but we may say that the diameter ranges between 1.5 and 2 μm.

The lamellar lipids are constituted by two components. The centre is occupied by a homogeneous material of irregular limits whose electron density reminds us of the lipid droplets described in the polymorphous dense bodies. Around this central component we find several concentric layers of a lamellar aspect. In most of the lamellar lipids we find apparently empty spaces, enlarged and having unclear limits, which are found at the level of the concentric layers. These spaces seem to be responsible for the irregular contour of the central component. In the periphery of the lamellar lipids, mainly the spaces between the lamina are occupied by cytoplasm in which we can sometimes distinguish cellular organelles (fig. 6). On numerous occasions we have been able to demonstrate that the peripheral lamellar component corresponds to cisterns deprived of ribosomes whose membranes have fused and the intracisternal space disappeared (fig. 6). These cisterns proceed from the rough endoplasmic reticulum, although, on rare occasions, we have found images which suggest the origin of the cisterns in the Golgi complex. These last images are mainly found in the lamellar lipids of smaller size, apparently in the process of formation.

The organelle, most frequently related to the lamellar lipids, is the rough endoplasmic reticulum. These organelles place themselves in the form of parallel cisterns, but they never surround the lamellar lipid nor do they place themselves concentrically to it. In the cisterns nearmost the lamellar lipids, we find images of membrane fusion like those described previously.

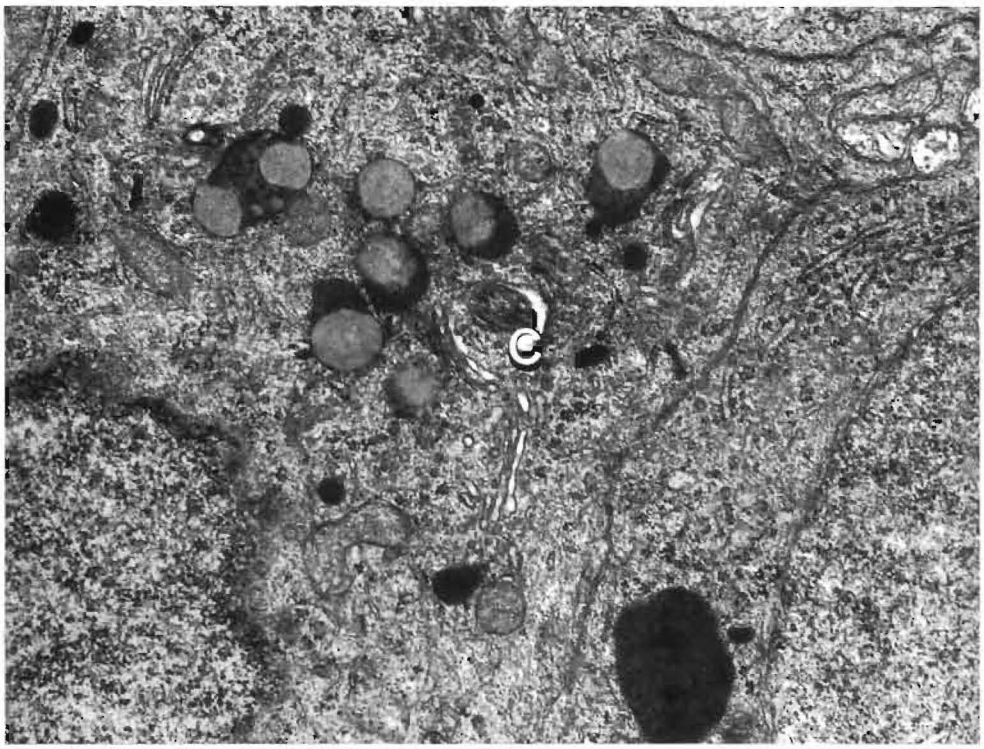
Less frequently, we found polymorphous dense bodies in contact with the lamellar lipids. However, we have not found images of fusion between both of these structures (fig. 8).

The B pinealocyte is a clearly polarized cell. This makes it possible to distinguish several regions in it, which we have previously described [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977a, b; *Calvo and Boya,* 1977a–d]. The soma and the supranuclear cytoplasm are very well developed, and therefore these cells have a typical globular aspect in the adult pineal. The neck contains, as in previous phases, numerous microtubules which – proceeding from the supranuclear cytoplasm – assemble here. The junctional mechanisms are located at this level (fig. 4, 10). The terminal club presents a aspect similar to that found in young animals, standing out the development of the ciliary processes (fig. 10), which appear swollen, and the whorl-like lamellar systems related to them. The ultrastructure, course and location of the basal process present no obvious differences in respect to younger animals (fig. 9).

The A pinealocytes are much more scarce than the B pinealocytes in the adult pineal. We may still observe the two clear and dense cell types of the A pinealocytes [*Boya and Calvo,* 1977a, b], although the differences in density between them have greatly diminished. Thus, we find that the dense A cell type is only

Fig. 11. 4-year-old hen. Dense type A pinealocyte. Dense bodies in relation to a lipid droplet in the vicinity of a Golgi complex. C = Cilium. × 15,000.

Fig. 12. 3-year-old hen. A pinealocyte. Positive reaction to acid phosphatase in the dense bodies (lysosomes). × 20,000.



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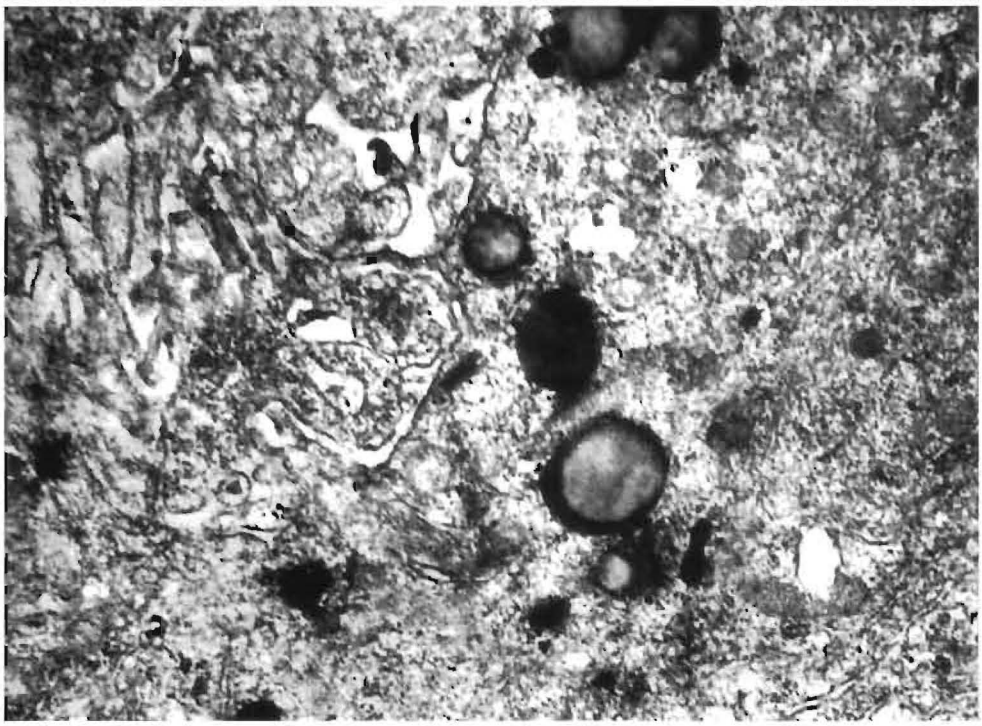

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slightly more electron-dense than the B pinealocyte. The clear cell type, more abundant than the dense cell type, still presents a cytoplasmic matrix which is almost electron-transparent. The rest of the ultrastructural characteristics is similar in both clear and dense A cell types.

The morphology and relations of the A pinealocytes show no important variations with respect to that described for younger animals [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977a, b; *Calvo and Boya,* 1977a–d]. The A pinealocytes have a scarce cytoplasm in comparison with the B pinealocytes, although is is more developed than in previous phases. The ovoid nucleus has a smaller size than that of type B. The chromatin appears more contrasted due to the lesser density of the nucleoplasm. It may present one or two nucleoli.

The A pinealocytes have a great relation to the pineal lumina. At this level, they present a cytoplasmic apical swelling which is very characteristic (fig. 10). The luminal surface of this cell presents short and little electron-dense microvilli as well as a cilium with a central pair of microtubules. The basal process heads towards the basal membrane (fig. 4), at which level it ends in an enlargement.

In the pineal of the adult chicken, the A pinealocytes show a cytoplasm that is broader and more rich in organelles than in previous phases. Among the organelles, the development of the Golgi complex and the presence of numerous round and small (0.2–0.4 μm) dense bodies are striking. They are located adjacent to the Golgi complex and in the vicinity of the luminal surface of the pinealocytes. In many cases the dense bodies appear fused to a single lipid droplet (fig. 11). This droplet, generally single, is surrounded by a thin surface of dense material. The lipid component is always much greater in size than the peripheral dense material.

The acid phosphatase technique, studied with the electron microscope, proves the presence of this hydrolase in the dense bodies of the A pinealocytes which, therefore, must be considered as lysosomes (fig. 12).

The rest of the organelles in the A pinealocytes is similar with respect to development and distribution to that described in young animals [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977a, b]. We may only emphasize the presence of 'nebenkern' in all the age intervals studied in adult life. In addition to the already formed 'nebenkern', we have found in the adult pineal images of the process of formation of these structures similar to those found in young chickens.

Discussion

As is demonstrated by our studies, the adult chicken pineal gland presents a characteristic morphology different to that observed in younger animals. In previous studies [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977a, b; *Calvo and Boya,* 1977a–d] we described the structure and ultrastructure of the chicken pineal along the embryonic development and until 13 months after hatching. With the present study we complete the description of the pineal histology of this species. Recently, *Omura* [1977] has studied the ultrastructure of the chicken pineal during its embryonic development and during post-hatching life until 2 months of age. However, his description is centred almost exclusively on the presence of the dense-cored vesicles in the B pinealocytes, paying little attention to the rest of the histological characteristics of the pineal gland.

The structural pattern of the adult pineal gland represents a more advanced stage in the

evolution which began in early periods of chicken life. The details of this evolution have been described previously [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977 a, b]. The evolution of the pineal structural pattern reflects mainly the transformation of the large follicular cavities into cellular rosette formations. In the centre of these formations, which appear compact under the light microscope, there is a cavity perfectly visible under the electron microscope. The larger cavities, which may be seen with the light microscope, correspond to the remains of the old follicular cavities which are thus still present in the adult pineal. The pineal cavities observed with the electron microscope appear occupied by the apical processes of the B pinealocytes. In all the age intervals studied, we frequently found whorl-like lamellar systems in the pineal lumina.

The arrangement of the pineal parenchyma in cellular rosettes is confirmed by the histochemical techniques for acid phosphatase which shows the pineal lysosomes arranged in circular formations of lysosomal 'rosettes'. Finally, the progressive penetration of stroma into the parenchyma fragments it into small territories composed of one or several cellular rosettes. As a final result of this evolution process in the adult pineal, every pinealocyte is in contact with a lumen and their somas are very near the basement membrane.

The tendency of the pineal to increase its number of cavities, so that each cell may be in contact with one of them, indicates that this organization pattern is important for pineal physiology and not merely a vestigial remembrance. This is also suggested by the clear polarization of the pinealocytes with respect to a central cavity. Also, the progressive penetration of the stroma would result in a greater contact between the parenchyma and the blood vessels and nerve fibres present in the stroma.

As shown by our ultrastructural results, the pineal gland is an active, functional organ during the adult life of the chicken. In effect, the cellular hypertrophy and the great development of the organelles in the pinealocytes, especially in type B, are the indication of a cellular activity. *Omura* [1977] also describes abundant organelles in this cellular type in 9-month-old chickens. The development of the rough endoplasmic reticulum, Golgi complex, as well as of clear and dense-cored vesicles related to the Golgi complex, suggests an increase in the secretory activity of the B pinealocytes. *Collin et al.* [1976] have suggested, in autoradiographical studies, the location of indoleamines in the dense-cored vesicles of the avian pinealocytes. The presence of the numerous mitochondria in the B pinealocyte would provide the necessary energy for an increased cellular activity.

From the morphologic point of view, the activity of the pineal gland must remain at a constant level during adult life. This may be demonstrated by the uniformity of the ultrastructural pattern of the pineal parenchymal cells in all the age intervals studied (1–5 years of age).

Finally, in none of the age intervals studied with the electron microscope we have found any images which indicate the appearance of degenerative or regressive phenomena in the pineal gland. The involution of the hen pineal gland must, therefore, take place in a stage above 5 years.

One the most characteristic organelles of the B pinealocytes is the polymorphous dense body [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977a, b; *Calvo and Boya,* 1977a–d]. In the adult pineal, these structures are much larger and more abundant than in the pineal

of young animals. Thus, this hypertrophy of the polymorphous dense bodies may be considered as a specific ultrastructural characteristic of the adult pineal. With the data available to us we cannot explain the significance of the hypertrophy of the polymorphous dense bodies.

In a previous paper [*Calvo and Boya,* 1977a–d] we demonstrated the lysosomal nature of the dense bodies in the B pinealocytes due to the presence of acid phosphatase in them. The hypertrophic dense bodies of the adult pinealocytes also present acid phosphatase activity, although the precipitate is not located in a uniform fashion in all the dense bodies. The presence of a reaction product for acid phosphatase located in an irregular fashion is usually found in secondary lysosomes. Morphologically, the polymorphous dense bodies of the adult pinealocytes do not present the typical heterogeneous aspect of these secondary lysosomes, although their hypertrophy could be a positive support for this idea.

The lamellar lipids are characteristic structures of the B pinealocytes. According to our results, they are present from the embryonic period until adult life. *Omura* [1977] describes a juxtanuclear lipid inclusion in pinealocytes of 21-day embryos. Its aspect corresponds to what we have named lamellar lipids. The detailed study of these structures demonstrates the formation origin of the peripheral lamellar component to be mainly the rough endoplasmic reticulum. A review of the material corresponding to younger animals confirms this idea.

We have found numerous images in the adult B pinealocytes which suggest the origin of the lamellar lipids to be the polymorphous dense bodies. The evolution process could follow a series of steps: (a) appearance of homogeneous droplets of small electron density, having a probable lipidic nature in the polymorphous dense body; (b) fusion of these droplets to constitute a single large one which occupies a great part of the dense body volume, and (c) placement of strands proceeding from the fusion of the membranes of the rough reticulum cisterns around the lipids and, thus, appearance of the lamellar lipid. We must, however, make clear the following points: (1) this explanation is based only on morphological observations, having no definite proofs of its validity in our material. (2) Certain aspects are not explained, such as the destiny of the dense material of the dense body or the negativity of the lamellar lipids to the techniques for acid phosphatase. We would also have to make clear why, although the number of polymorphous dense bodies is very abundant, we only found one or two lamellar lipids per cell. (3) This diagram can only be applied to adult pineals since the polymorphous dense bodies of pineals of young animals do not present lipid droplets. Further investigations are necessary in order to proof this hypothesis and to obtain data about the formation of lamellar lipids in embryos and young animals.

The A pinealocytes during adult life are large cells rich in organelles with respect to those observed in younger animals. This cellular type has been regarded as a supporting cell by several authors [*Oksche and Vaupel von Harnack,* 1966; *Fujie,* 1968; *Collin,* 1971; *Omura,* 1977] and also as an ependymal cell [*Bischoff,* 1969]. The function of mechanical support, if such, must not be very important if we are to judge by the amount of microtubules and microfilaments present in this cellular type. Instead, its relation to ependymal cells is more evident. In effect, the study of the choroid epithelium reveals the presence in its

cells of round dense bodies, often related to a lipid droplet, similar to those present in A pinealocytes. Also, the microvilli, typical of the A pinealocyte, are similar to those of the choroid epithelium. Finally, the cells of the choroid plexus present cilia as in the A pinealocytes.

In previous papers [*Boya and Zamorano,* 1975; *Boya and Calvo,* 1977a, b; *Calvo and Boya,* 1977a–d] we have described the relation between the A pinealocytes and the follicular lumina in formation. In the adult pineal, the development of organelles in these cells could indicate a metabolic or nutritional function of the A pinealocyte.

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