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The influence of sex hormones on the pineal gland of the chick: A histochemical and ultrastructural study

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INTRODUCTION

The first function attributed to the pineal gland is its influence over the gonads, a function which has been extensively documented. However, there are few studies dealing with the influence of sex hormones upon the pineal gland, although the possibility of this influence has been indicated in the studies of Goodyear (1972), Nagle, Cardinali & Rosner (1972, 1973) and Cardinali, Nagle & Rosner (1974, 1975), which show an intense uptake of radioactive oestrogen and testosterone by the pineal gland.

Research dealing with the influence of sex hormones upon the pineal gland has been performed mostly in rats, and studies have been mainly biochemical (Alexander, Dowd & Wolfson, 1970*a*; Nir, Kaiser, Hirschmann & Sulman, 1970; Weiss & Crayton, 1970*a*, *b*; Houssay & Barcelo, 1972*a*, *b*; Nagle *et al.* 1972, 1973, 1974). The results obtained have not been conclusive, and they have frequently been contradictory.

In birds, variations in hydroxyindole-0-methyltransferase (HIOMT) activity have been described during the process of sexual maturity (Alexander, Dowd & Wolfson, 1970*b*; Preslock, 1975). According to Preslock, castration caused a decrease in HIOMT activity in the pineal gland of coturnix quails of both sexes; the administration of sex hormones to the castrated animals restored the levels of HIOMT. An increase in HIOMT activity was found in coturnix quail after the administration of oestradiol and testosterone (Preslock, 1976).

In previous studies important changes were found in the arrangement and ultrastructure of the pinealocytes during the process of sexual maturity in the chick (Boya & Calvo, 1979*b*) which were still found in adult life until the most advanced age studied (Boya & Calvo, 1979*a*). The pineal changes which appear during the process of sexual maturity in the chick may thus be due to increases in circulating sex hormones, and the present study deals with the pineal variations found after the administration of sex hormones to immature chicks. The techniques used were electron microscopy and the acid phosphatase histochemical technique which, as previously demonstrated, clearly shows variations in chick pineal architecture (Calvo & Boya, 1979*b*).

MATERIALS AND METHODS

The study was performed on chicks (*Gallus gallus*) which were maintained from post-hatching onwards in natural conditions of light and feeding. The animals were

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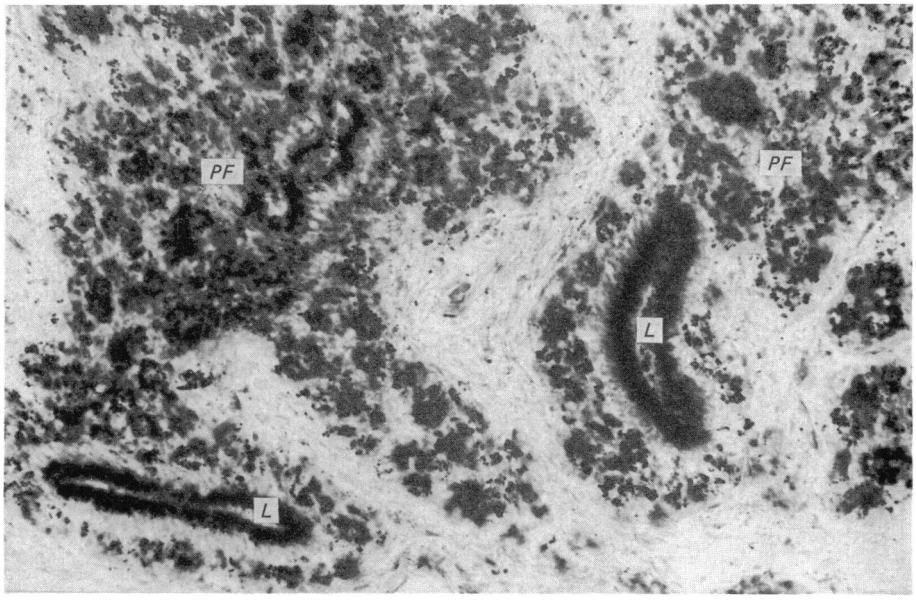

Fig. 1. Pineal follicles present large lumina (*L*) limited by lysosomic bands of the pinealocyte follicular layer. In the parafollicular (*PF*) layer the lysosomes appear in rosette formations. 45 days. Control. Acid phosphatase technique. × 140.

divided into three groups, two of which were treated with oestrogens and androgens respectively, while the third served as control.

The hormones used were oestradiol benzoate and testosterone propionate, each administered for three days at doses of 0·5 mg and 2·5 mg respectively. The administration was performed i.m. beginning from the first day post-hatching. The controls were given the same solvent dose.

The animals were killed at 30, 45, 60, 75 and 90 days post-hatching, five animals being taken from each of the three groups at each of these dates. The histochemical studies were made on pineals fixed in 0·1 M cacodylate-buffered 3 % glutaraldehyde at pH 7·4, for 2 hours at 4 °C. After washing the samples in cacodylate buffer, sections were obtained with a freezing microtome and incubated for 30–45 minutes at 37 °C in Miller & Palade's (1964) modification of the Gomori medium for acid phosphatase.

For the ultrastructural study the pineals were fixed by immersion in 0·1 M phosphatase-buffered 3 % glutaraldehyde, at pH 7·4 at 4 °C. They were post-fixed in phosphate-buffered 1 % osmium tetroxide. After dehydration in acetone, they were embedded in Epon 812 (Luft, 1961). The sections obtained from an LKB ultramicrotome were stained with uranyl acetate and lead citrate and examined with an EM 201 Philips electron microscope.

RESULTS

Histochemistry

The histochemical technique for acid phosphatase showed no prominent quantitative variations. However, variations were found in the distribution pattern in pinealocyte lysosomes and from 45 days onwards these differences were clear. Control

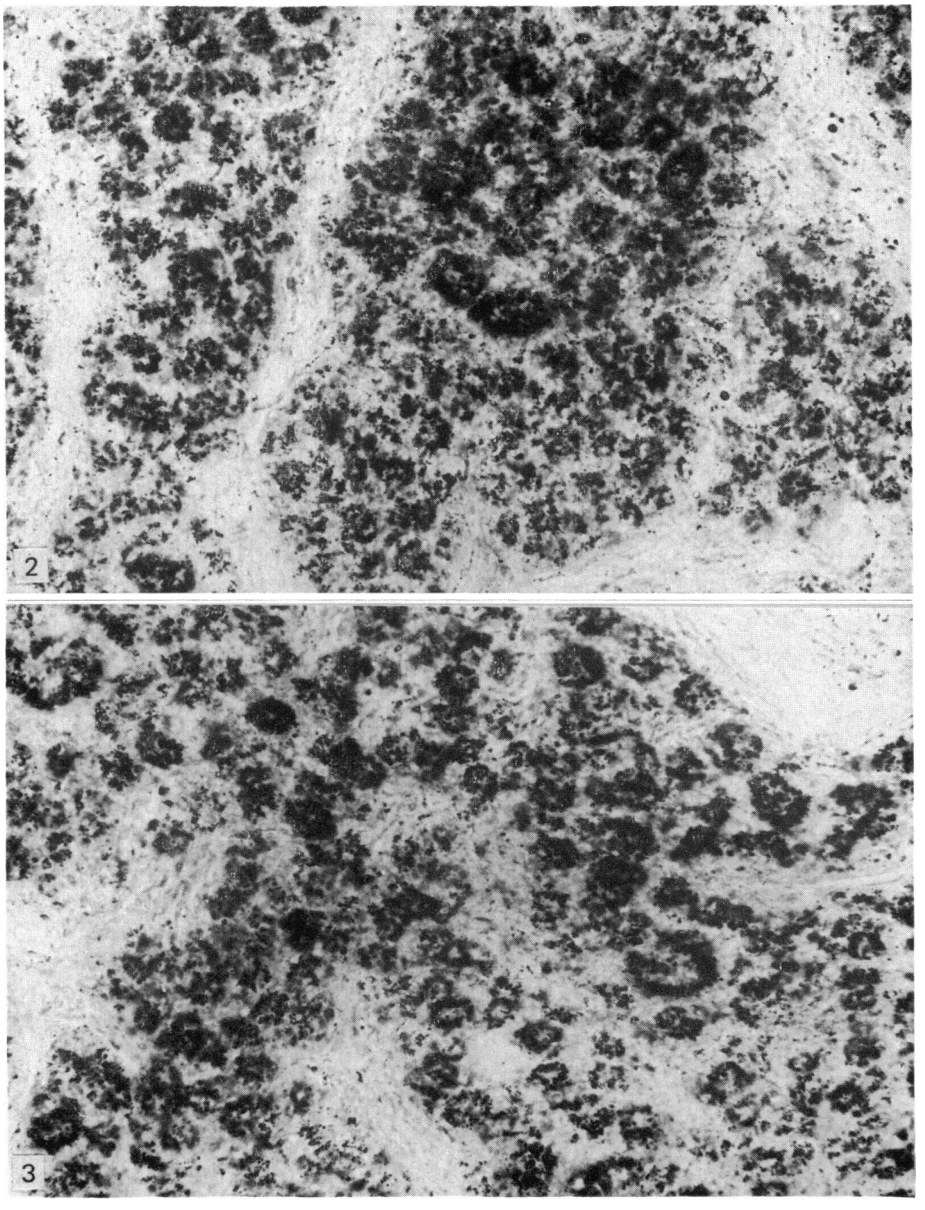

Fig. 2. The large follicular lumina have disappeared. Lysosomes mainly form rosettes. 45 days. Oestrogens. Acid phosphatase technique. × 140.

Fig. 3. The large follicular lumina have disappeared. Lysosomes mainly form rosettes. 45 days. Androgens. Acid phosphatase technique. × 140.

animals (Fig. 1) still presented large follicular lumina which showed bands of acid phosphatase-positive granules (lysosomes) belonging to the pinealocytes of the follicular layer. In the parafollicular layer the lysosomes were arranged in small bundles forming 'rosettes'. In the animals treated with androgens (Fig. 3) as well as with oestrogens (Fig. 2), the large follicular lumina had disappeared and only their remains could be found. Lysosomes were mainly found forming multiple 'rosettes',

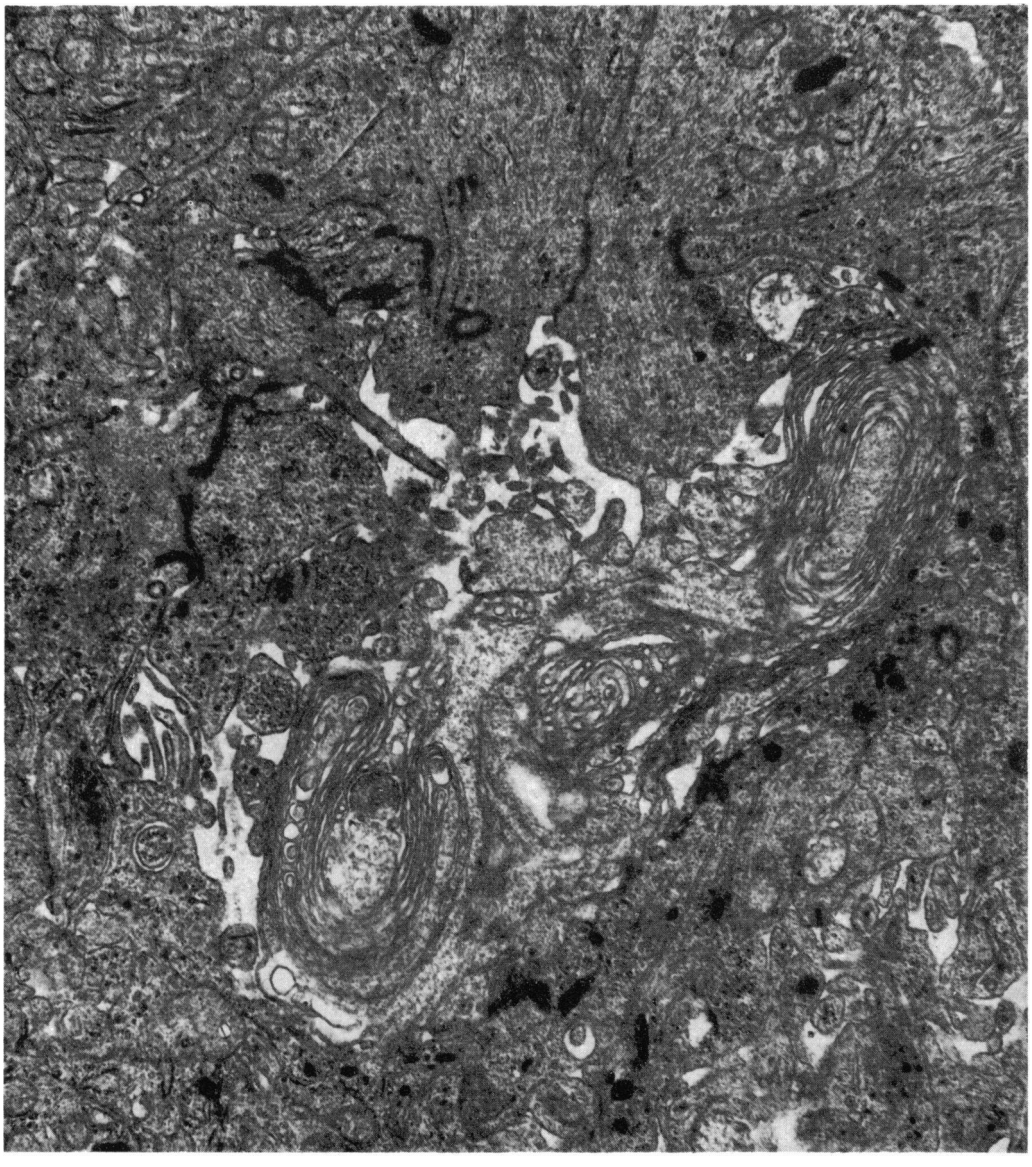

Fig. 4. A small lumen is occupied mainly by pinealocyte apical projections and lamellar whorls. 45 days. Oestrogens. ×13500.

leaving a small central space such as those present in the parafollicular layer of control animals.

Ultrastructure

The ultrastructural variations in the pineal gland of the animals treated with hormones were of the same type in all the age intervals studied, these variations being more evident in the pineals of longer treatment. Therefore the ultrastructural variations will be described as a whole in each of the experimental groups.

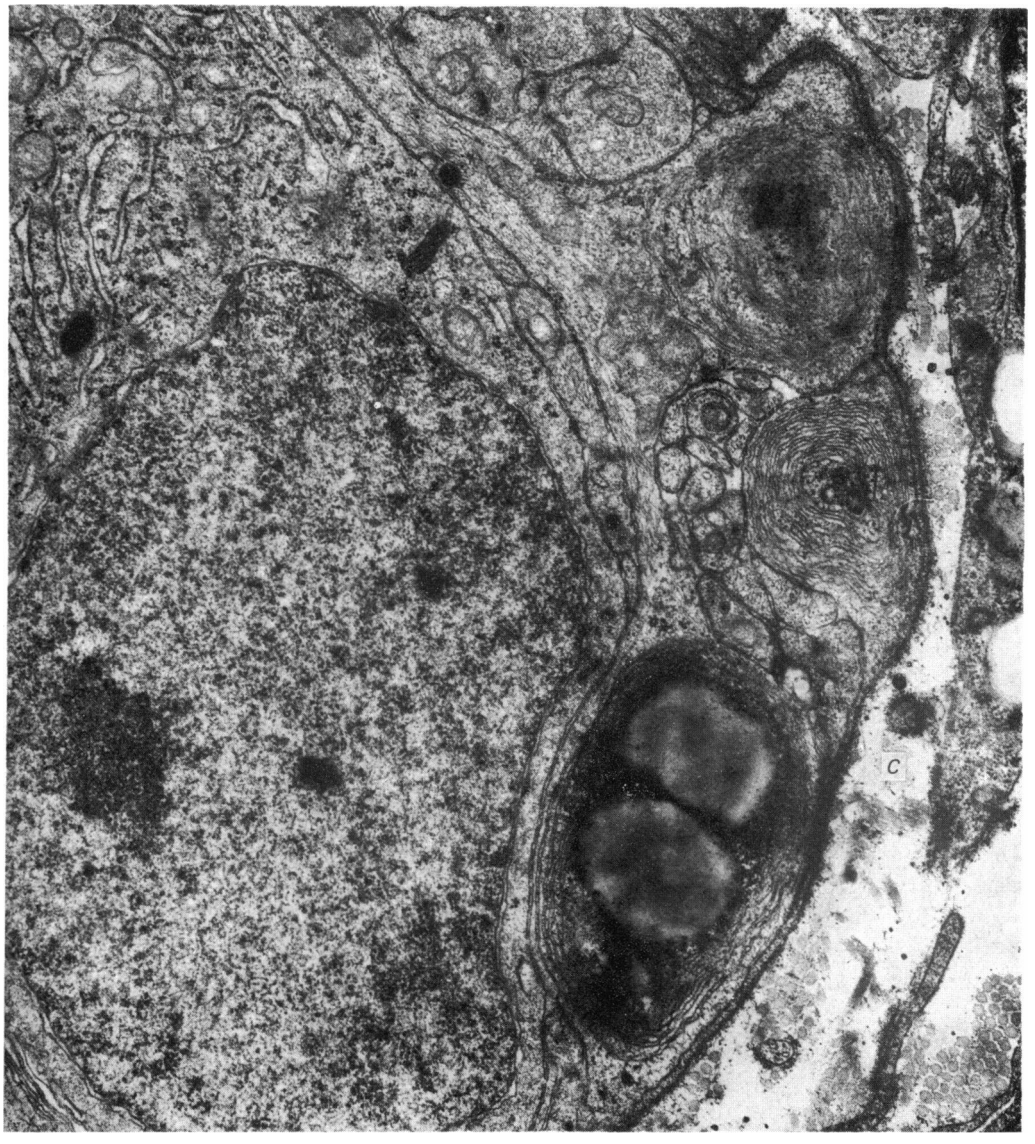

Fig. 5. To show the boundary between pineal parenchyma and connective septum (*C*). Nebenkern are present in A pinealocyte basal processes. Two of these show a central lipid component. 60 days. Oestrogens. × 19500.

Oestrogens

Practically all the changes found in the oestrogen-treated pineals were located in the parenchyma which, compared to the control animals, showed a more compact form. Abundant small lumina were almost completely occupied by the apical projections on the pinealocytes. The appearance of lamellar systems in these lumina (Fig. 4) must be emphasized; they appeared in the oestrogen-treated chicks at 45 days of age and increased in later stages.

In all stages of oestrogen treatment, the pinealocytes presented larger volumes of

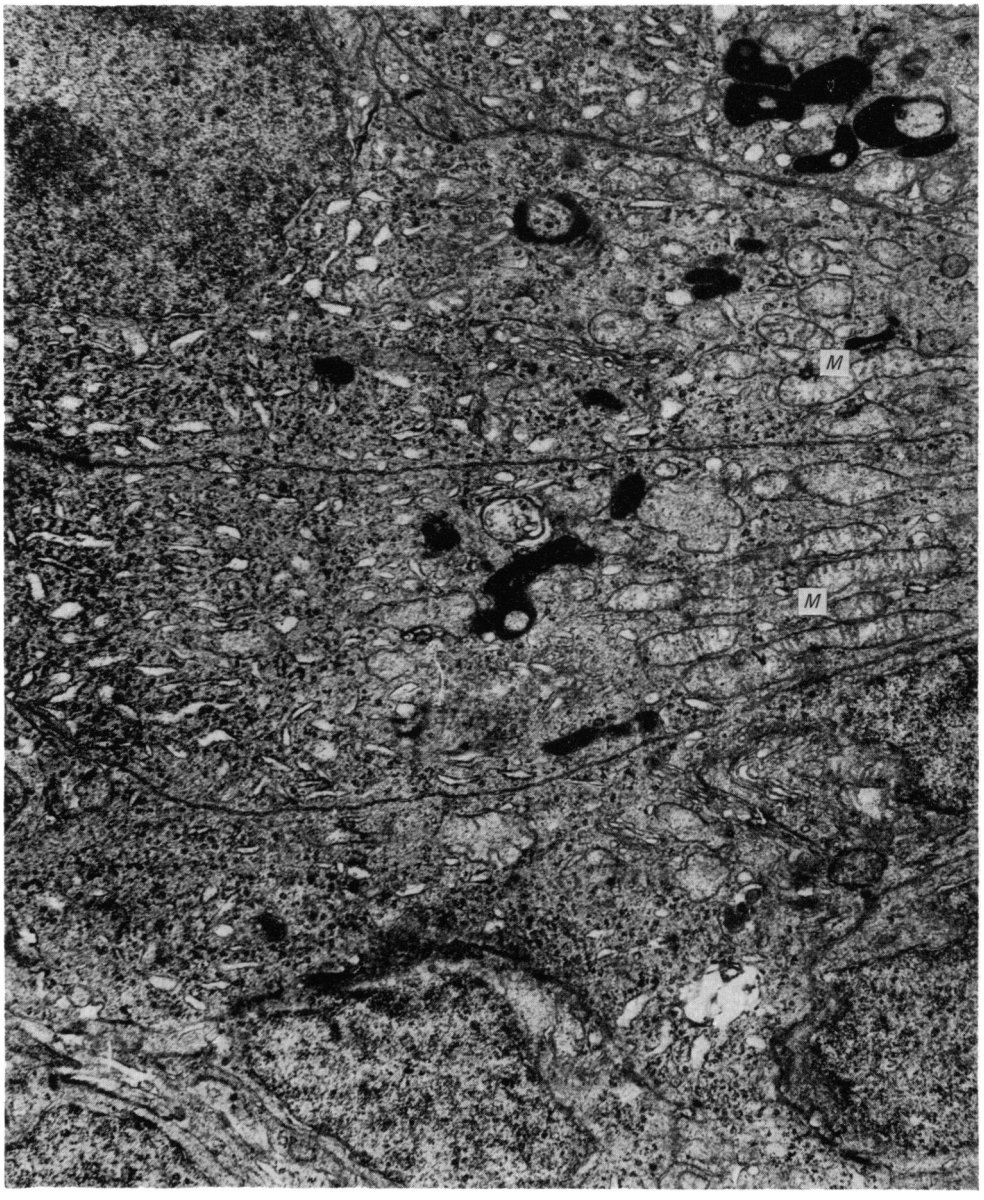

Fig. 6. Pinealocytes of type B show abundant polyribosomes and moderately widened rough endoplasmic reticulum. There are also abundant supranuclear mitochondria. 75 days. Oestrogens. × 13500.

cytoplasm with more abundant organelles than did the controls of the same stage, and hypertrophy was more evident with the passage of time.

There were no important differences in the ultrastructural appearance of the A pinealocytes. The most distinctive feature was the appearance of Nebenkern at 60 days of age (Fig. 5) in the oestrogen-treated chicks.

The B pinealocytes presented a large volume of cytoplasm rich in organelles. In the perinuclear cytoplasm cisterns of round endoplasmic reticulum and abundant

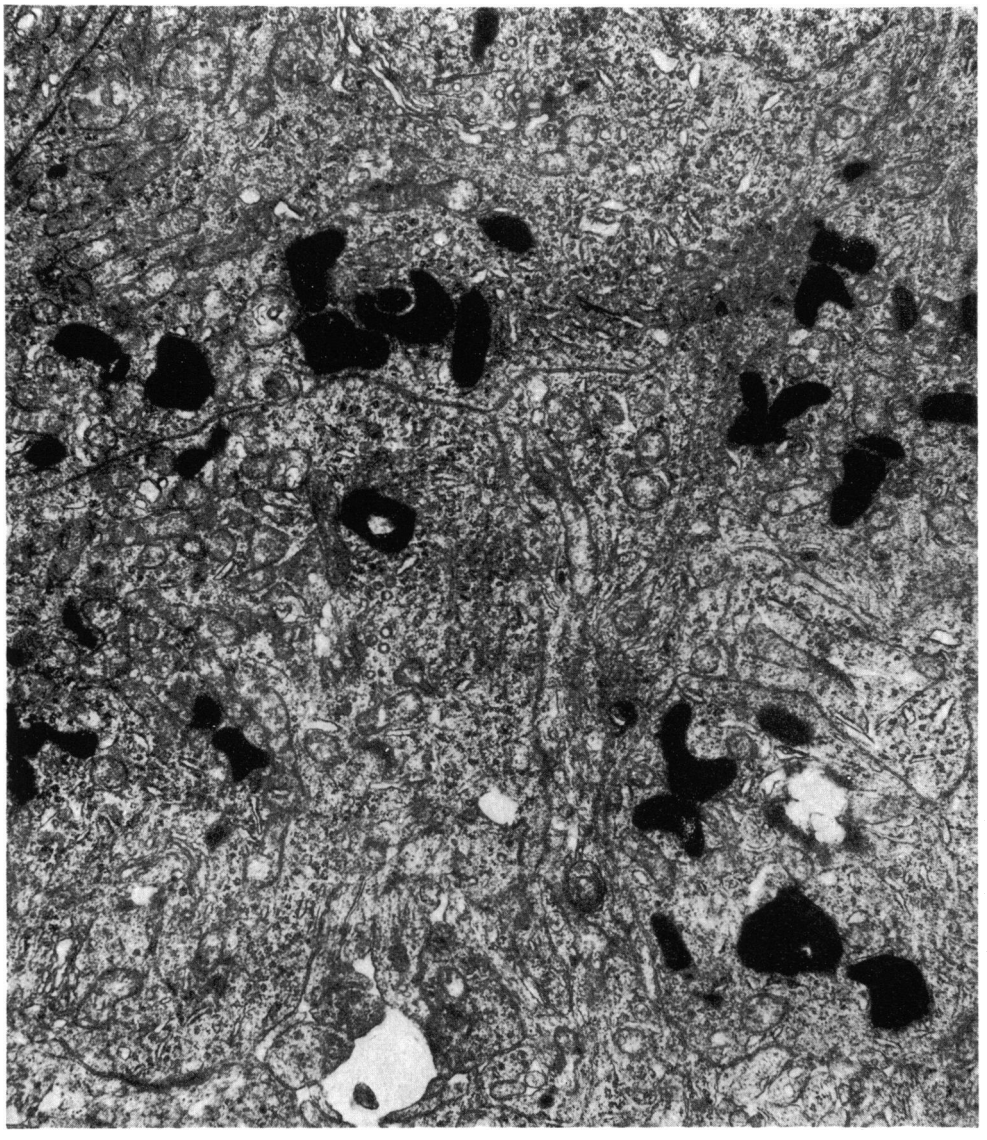

Fig. 7. The cytoplasm of a B pinealocyte shows abundant polymorphous dense bodies. 75 days. Oestrogens. × 13500.

free ribosomes were present (Figs. 6, 8), together with a well developed Golgi system. However, the most characteristic ultrastructural feature was the abundance of mitochondria in the apical cytoplasm of the B pinealocyte (Figs. 6, 8). There was an increase in the number and size of the polymorphous dense bodies, which are the lysosomes of the B pinealocyte (Calvo & Boya, 1979) (Figs. 7, 8). Finally, there was a decrease in the diameter as well as in the amount of lamellar lipids of the B pinealocytes as compared to the controls. In respect to the nucleus, the development of the nucleoli was striking.

Above the basement membrane of the pineal parenchyma a layer rich in basal processes was found. These processes showed abundant clear vesicles and synaptic

Fig. 8. Apical cytoplasm of B pinealocytes limits a lumen (*L*). Arrowheads, polymorphous dense bodies. 75 days. Control. ×15680.

ribbons, both being more abundant than in the controls. There was also a less prominent increase in the amount of granular vesicles.

Androgens

The pineal parenchyma of the androgen-treated chicks also showed a compact form. Large lumina could not be found but, instead, numerous small lumina were scattered in the parenchyma and were occupied by apical processes of the B pinealocytes (Fig. 9). Lamellar whorls were rarely found there. There also seemed to be a reduced development of the B pinealocyte cilia.

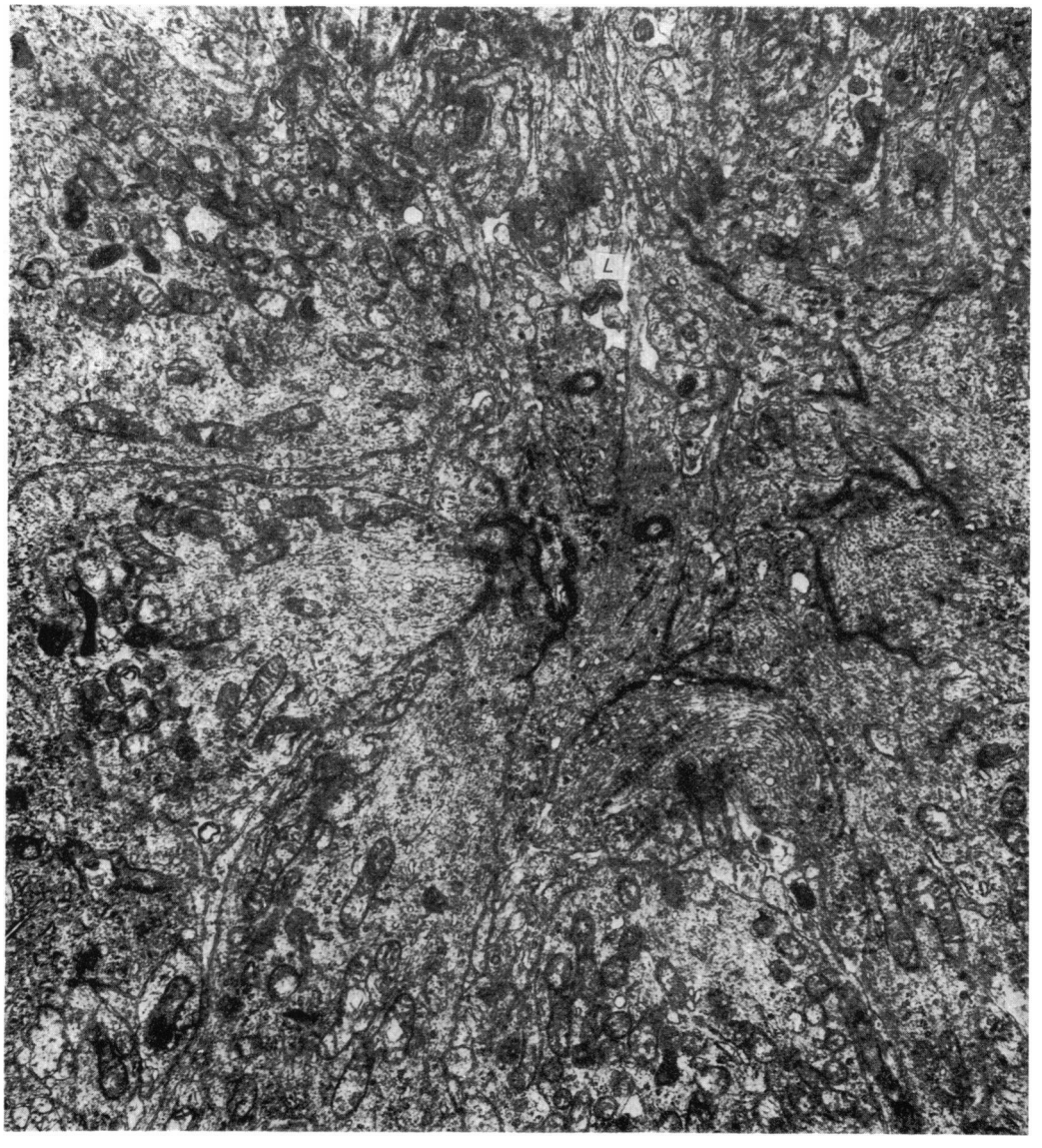

Fig. 9. A small follicular lumen (*L*) is occupied by B pinealocyte apical projections. Apical cytoplasm of B pinealocytes contains abundant mitochondria. 60 days. Androgens. × 13500.

Type A pinealocytes showed no evident variations. Dense A pinealocytes were very frequent and Nebenkern did not occur in the androgen-treated chick pineal.

The B pinealocytes presented a large volume of cytoplasm, as described also for the oestrogen-treated pineals. However, there were differences in respect to the development of the various organelles. In the perinuclear cytoplasm of the androgen-treated pineals, free ribosomes were more abundant than rough endoplasmic reticulum. The Golgi system showed a great hypertrophy, but the polymorphous dense bodies resembled those found in the control animals. The lamellar lipids were small and scarce although somewhat more abundant than in the oestrogen-treated

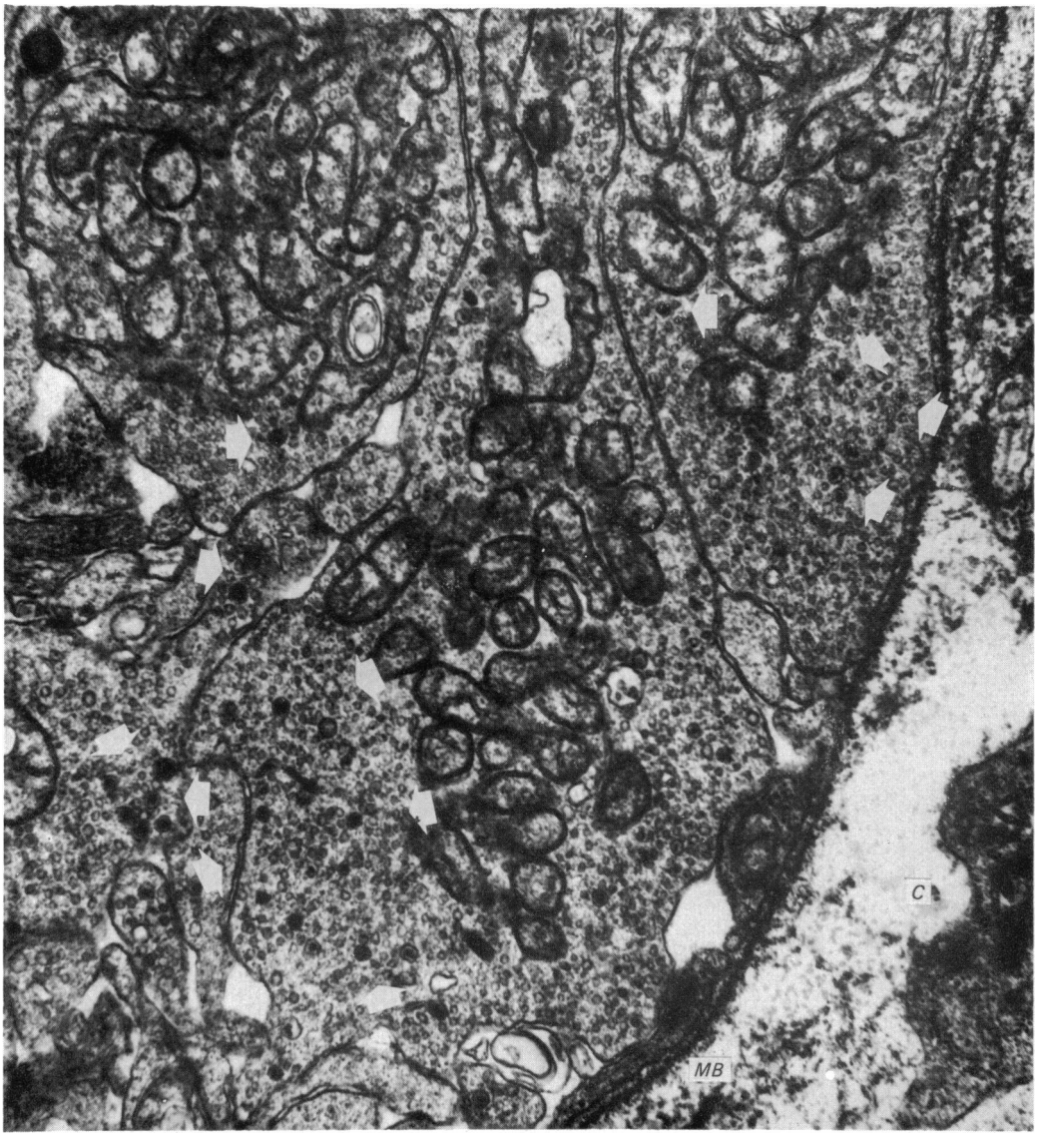

Fig. 10. The basal processes of B pinealocytes show abundant clear vesicles among which are granular vesicles (arrowheads). The connective space (C) is separated from the processes by the basal membrane (MB). Note the clusters of mitochondria in these processes. 75 days. Androgens. ×26500.

pineals. Finally, the apical cytoplasm of the B pinealocytes presented abundant mitochondria.

In comparison to the controls, the basal processes of the B pinealocytes were larger and contained more numerous clear vesicles, while synaptic ribbons were scarce. However, there was an increased amount of granular vesicles in the B pinealocyte processes (Figs. 10, 11). It is important to point out that this increase was also found in the cell body, where vesicles of this type were numerous (Fig. 12). These vesicles were rare in the controls as well as in the oestrogen-treated pineals. A peculiar feature of the androgen-treated pineals was the appearance of widened

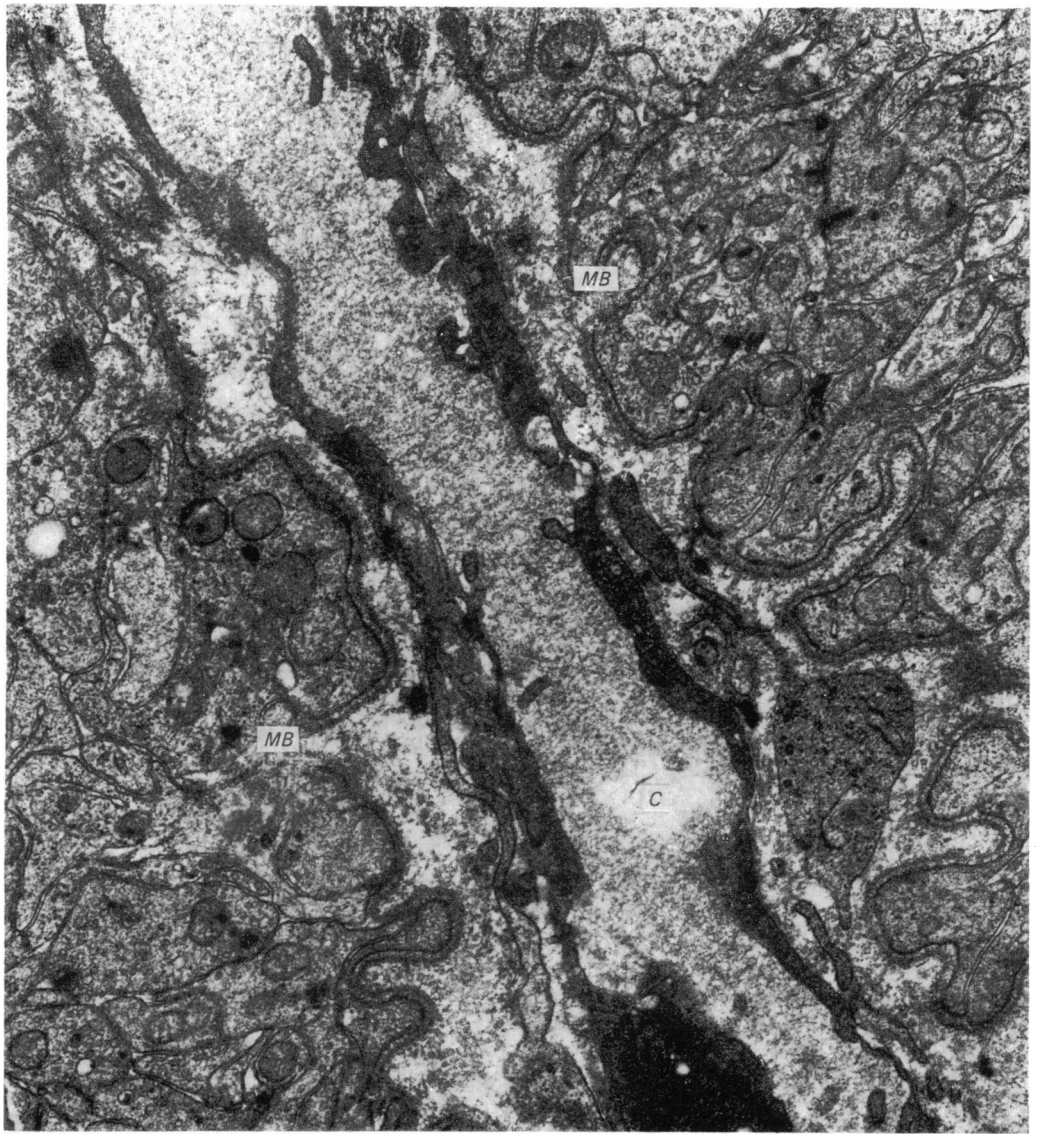

Fig. 11. To show the basal area of the pineal parenchyma which mainly contains basal processes of the B pinealocytes. *MB*, basement membrane; *C*, capillary. 75 days. Control. × 20800.

processes full of mitochondria situated in the vicinity of the basal membrane. The location and aspect of these formations and the morphology of the nearby parenchyma eliminated the possibility of their being secretions of apical cytoplasms of B pinealocytes.

Frequently the B pinealocyte dense bodies of the androgen-treated chicks presented round zones of lesser electron density and variable size, whose ultrastructural aspect resembled lipid droplets (Fig. 13). Once a certain size had been reached these droplets occupied most of the volume of the dense body, forming structures similar to those described for adult animals (Boya & Calvo, 1979*a*) in relation to the evolution of dense bodies into lamellar lipids. However, this evolution did not seem to be

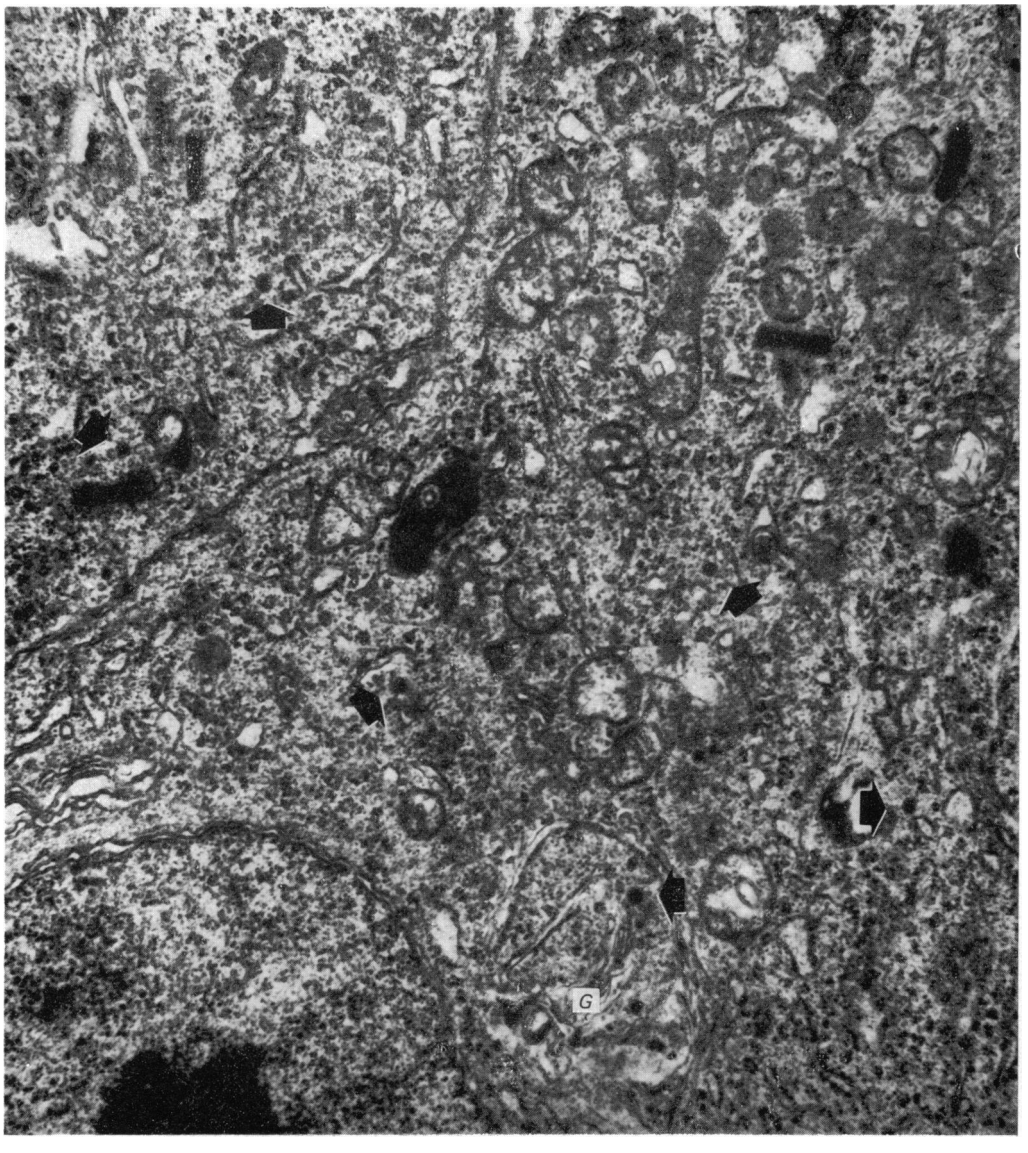

Fig. 12. The cell body of a B pinealocyte shows granular vesicles (arrowheads) sometimes related to the Golgi system (*G*). 75 days. Androgens. × 19500.

complete in the time stages studied for the androgen-treated animals; fully modified dense bodies were not seen (Boya & Calvo, 1979*a*). In the control animals, the polymorphous dense bodies of the B pinealocyte were homogeneous, and clear areas in their interior were only rarely found (Fig. 8).

DISCUSSION

Our results have shown the occurrence of morphological changes in the chick pineal gland associated with sex hormone treatment which seem to indicate an early maturity of the gland. In effect, the pineal parenchyma of immature chicks is similar

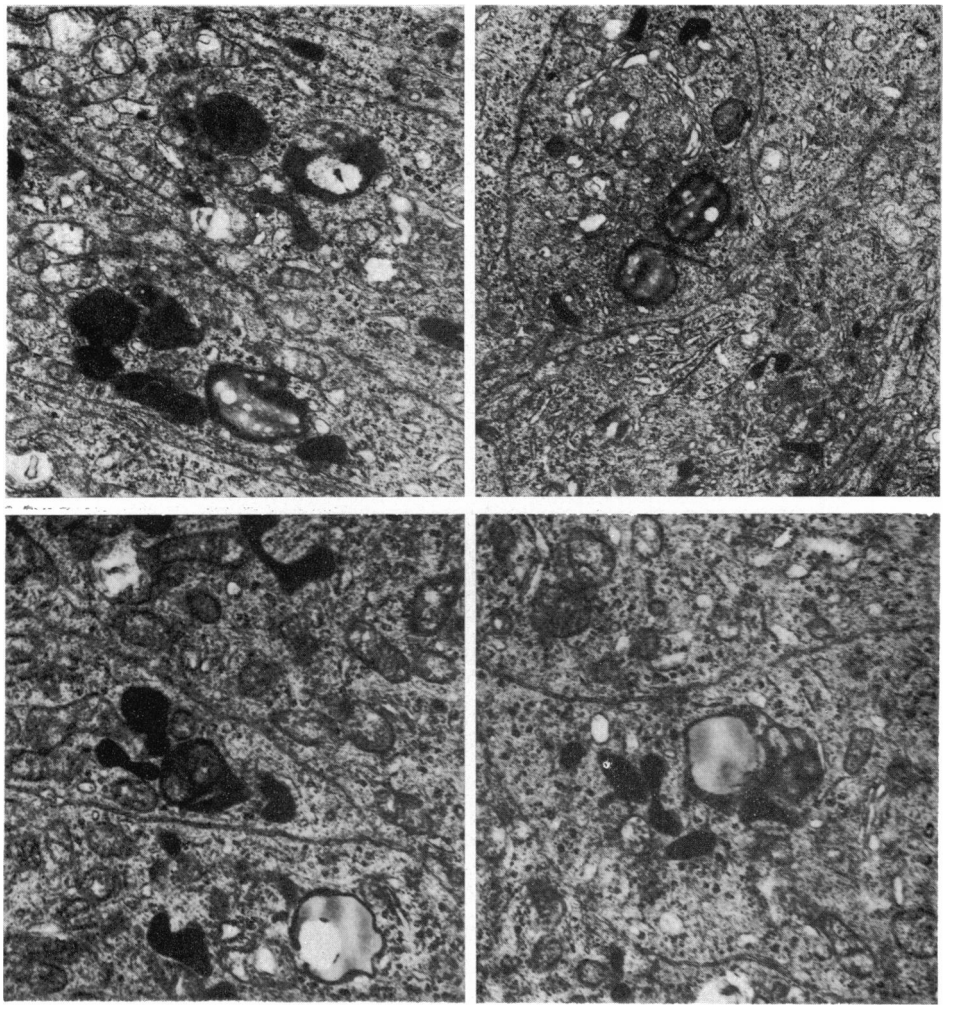

Fig. 13. To show polymorphous dense bodies with lipid components of different size and shape in B pinealocytes. 75 days. Androgens. ×16000.

to that previously described for adult animals (Boya & Calvo, 1979*a*, *b*). In adult chicks the pineal parenchyma presents a compact mass due to the disappearance of the large follicular cavities which are characteristic of the embryonic period and the first month's post-hatching (Calvo & Boya 1978; Boya & Calvo, 1978). The pinealocytes are placed radially, in 'rosette' formation, around small lumina visible only with the electron microscope. The polymorphous dense bodies, or B pinealocyte lysosomes (Calvo & Boya, 1979), are also placed radially in respect to the lumina. Thus, they too appear in a 'rosette' formation. The chick pineal gland reaches an adult pattern around eight months of age, remaining so during adult life. In the hormone-treated animals, the pineal lysosomes had adopted the 'rosette' formation from 45 days onwards. At 75 and 90 days their appearance was similar to that described for adults (Boya & Calvo, 1979*a*). Ultrastructurally, the early evolution of the treated chick's pineal gland has been demonstrated by its compact parenchyma, lacking large follicular cavities. Instead, numerous small lumina were found, occu-

pied by apical pinealocyte projections. This pattern corresponds to the lysosomic 'rosettes' which were observed using the technique for acid phosphatase. The pineals of immature chicks treated with sex hormones also showed numerous cytological characteristics (cellular hypertrophy, development of dense bodies, appearance of lamellar systems, Nebenkern, etc.) previously found only in adult animals (Boya & Calvo, 1979*a*). A previous study (Boya & Calvo, 1979*b*) suggested that the pineal changes which take place during the process of chick sexual maturity could be due to a normal increase in circulating sex hormones. In the present study the administration of these hormones to immature chicks (in order to produce an early increase of sex hormones in blood) appears to confirm this suggestion.

The early maturity of the gland has been found in chicks treated with androgens as well as with oestrogens, and since all our observations have been made on male animals, the results obtained seem to indicate that the action of sex hormones in the pineal gland is not totally specific. However, two observations must be made. Firstly, the hormones were administered immediately post-hatching and at high doses, and under these two conditions it is possible that the male chick pineal could become sensitive to administered oestrogens. Secondly, although there was an early maturity of the gland in both experimental groups, the ultrastructural differences found between them might indicate a differential action of the two hormones. In the case of androgen administration there was a considerable increase in the number of granular vesicles (dense granules) in the cytoplasm of the B pinealocytes, both in the cell body and in the basal process. There was also a greatly developed Golgi system and an increase in the amount of rough endoplasmic reticulum. Finally, clusters of mitochondria were frequently found in the basal process. All these changes suggest an increase in the synthesis and probably in the release of the content in the B pinealocyte granular vesicles. Recently, various authors have described the existence of peptide pineal substances having an antigonadotrophic function (Quay, 1974; Benson, 1977). On the other hand, the nature of the content in the dense granules of the parakeet has been demonstrated to be peptidic (Juillard & Collin, 1978). Thus the increase in the amount of granular vesicles in the B pinealocyte after androgen treatment may be related to an increase in the synthesis and release of these antigonadotrophic peptide pineal products.

The presence of lamellar whorls and the appearance of Nebenkern in the A pinealocytes stood out among the changes found in the oestrogen-treated animals. According to previous descriptions (Boya & Calvo, 1979*a*) these structures are abundant in the pineal of adult hens and, therefore, their appearance after oestrogen treatment is meaningful. Another important feature in the B pinealocyte which is also found in the pineal of adult hens is the increase in the number and volume of the polymorphous dense bodies (Boya & Calvo, 1979*a*). However, instead of an increase in the lamellar lipids, these structures were found to be less developed than in the control animals. This could reflect an increase in the renewal of these structures, possibly provoked by the administration of sex hormones. Due to the lipid solubility of melatonin the pinealocyte lipid inclusions have been considered important structures, although the content of melatonin or other indoles in them has not yet been demonstrated. A biochemical determination of the levels of melatonin and pineal peptides in the oestrogen- and androgen-treated chicks would be very interesting.

SUMMARY

The pineal gland of chicks treated with oestrogens and androgens has been studied histochemically and ultrastructurally from post-hatching until 90 days of age. The results obtained may demonstrate a precocious maturity of the gland caused by its response to the early high level of sex hormones circulating in the blood.

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