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Changes observed in the median eminence, the adenohypophysis and the thyroid gland in *Rhacophorus leucomystax maculatus* (Gray) during metamorphosis

by

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Most anuran amphibians begin life as tadpoles which are free living aquatic forms. These are subsequently transformed by a process of extensive morphological change into adult forms that usually lead a terrestrial existence. This transformation takes place in an orderly fashion, each new development bringing the animal closer to its adult form. The first change is the development of hind limbs followed, after a period of about 4 to 5 weeks, by the appearance of forelimbs and this phase is referred to as the prometamorphic period. When the hind limbs have grown to the size of its body, the tadpole enters a period of rapid growth called the metamorphic climax. During this period the forelimbs erupt and the lungs develop while the gills and tail are resorbed. By the end of the week of metamorphic climax the animal is ready for its terrestrial existence.

The central role of the hypophysis-thyroid axis in regulating amphibian metamorphosis has been established (Etkin, 1964). The metamorphic changes in the tissues are activated by the thyroid hormone, the activity of the thyroid gland in turn being controlled by thyroid stimulating hormone (TSH) secreted by the cells of the adenohypophysis. The activity of the cells of the adenohypophysis, secreting TSH, is under the control of a neurosecretory mechanism originating in the hypothalamus. Axons of certain hypothalamic nuclei, carrying specific neurosecretory material, terminate in the median eminence where a network of capillaries is found. From these capillaries originate the hypophyseal portal vessels that carry the neurosecretory material from the median eminence to the sinusoids of the adenohypophysis.

Arsecularatne, de Silva, Bandunatha, Tennekoon, Wijesundera and Balasubramaniam (1969) have reported that when *Rhacophorus leucomystax maculatus* tadpoles were treated with sublethal doses of aflatoxins, there was a delay in the appearance of limbs and in metamorphosis when compared with those in the control group. To determine the probable site at which aflatoxins may act to bring about such retardation in metamorphosis, a knowledge of the normal morphogenesis of the median eminence, the adenohypophysis and the thyroid gland was considered essential. A perusal of the literature on amphibian metamorphosis did not reveal any data on the duration and stages of metamorphosis and the morphogensis of these three strucures in *Rhacophorus leucomystax maculatus* and this study was undertaken to establish these norms.

MATERIALS AND METHODS

Tadpoles from a single spawn nest were obtained and bred singly in earthenware pots containing tap water. The pots were placed in the open to simulate as far as possible a normal environment of the species. The food consisted of parboiled spinach and the water in the pots was changed at regular intervals to prevent formation of slime which has been reported to be toxic to the animals (Arsecularatne, 1969).

Measurements were done at weekly intervals. This was carried out by placing the tadpole in a petri dish containing water. The dish was placed over a scored cardboard and the lengths measured in millimeters. The body length and the total length, including the tail, were measured in each animal. The following tadpoles were taken to study the morphogenesis of the median eminence, adenohypophysis and the thyroid gland.

Body length/total length indicated as BL/TL.

Group A BL/TL 12/30
Group B BL/TL 17/35
Group C BL/TL 17/57 -- one day after appearance of hind limbs
Group D BL/TL 18/50 — one day after appearance of forelimbs
Group E BL/TL 18/45 — two days after appearance of forelimbs

4 animals comprised each group. Each animal was measured before fixing in Bouin's fluid, embedded in paraffin and serially sectioned at 10μ thickness. All sections were stained in Delafield's haematoxylin and eosin. The remaining tadpoles were allowed to proceed with metamorphosis to determine the time taken for the metamorphic climax.

RESULTS

The prometamorphic period lasted from 3 to 4 weeks. The metamorphic climax lasted three days and in this it differred from other anurans described by Etkin (1964) where metamorphic climax lasted seven days. This difference is perhaps due to the higher temperature of the environment of tadpoles of *Rhacophorus leucomystax maculatus*.

Changes seen in the thyroid gland.

The thyroid gland appeared as a paired structure on either side of the midline in the pharyngeal region, anterior to the fused ceratohyal and hypohyal cartilages. The hyoid muscle was lateral and the ventral carotid arteries were observed ventral to the gland. The gland was covered with a fibrous capsule. Observations were made as regards the size of the gland, the shape and the size of follicles, the nature of the lining epithelium of the follicles and the distribution of colloid within the follicles. The volumetric size of the gland was measured with a graticule; the area occupied by thyroid tissue in each section was measured in square microns and the volume obtained by adding the areas in sections in which thyroid tissue was observed and then multiplying by a factor of 10, which was the thickness, in microns, of each section. Since all the specimens were processed in a similar manner, no correction was made for tissue distortion. These measurements were made to assess the relative sizes of the organs concerned. The measurements are tabulated in Table 1.

TABLE 1 — Volume of thyroid gland expressed in 10^6 cu μ.

TADPOLE	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
1	0.0088	1.0143	26.1954	29.7363	29.4058
2	0.0044	0.9938	24.8718	29.5514	29.5690
3	0.0041	1.0332	25.1772	29.4147	27.9631
4	0.0082	0.9875	25.3723	30.8273	28.4522
	No limbs	No limbs	Hindlimb Appeared	Forelimb 1 day	Forelimb 2 days

Group A — BL/TL 12/30

A single thyroid follicle, containing colloid, lined by flattened squamous cells was observed in transverse sections of the animal. The nuclei of these cells showed condensed chromatin (Fig. 1).

Group B — BL/TL 17/35

About 5 to 6 follicles were seen on an average in most transverse sections. The lining epithelium of the follicles were cuboidal in nature. Some of the nuclei showed condensed chromatin while in others the nuclei were lightly stained. Colloid appeared, in sections, as a solid acidophilic mass filling the entire follicle (Fig. 2).

Group C — BL/TL 17/57 — *one day after appearance of hindlimbs*

The thyroid was larger in size and consisted of follicles which were rounded or ovoid in cross section. The follicles were lined by cuboidal epithelium and the colloid did not completely fill the lumen of the follicle (Fig. 3)

Group D — BL/TL 18/50 — *one day after appearance of forelimbs*

The individual follicles were larger in size, the lining epithelial cells of the follicle columnar and the nuclei of these cells well stained, with prominent nucleoli. The colloid in the follicle was retracted from the lining cells thereby giving a serrated border to the colloid. A large number of capillaries was another noteworthy feature observed in the substance of the gland (Fig. 4).

Group E — BL/TL 18/45 — *two days after appearance of forelimbs*

The thyroid follicles, in section, appeared to be very similar to those of Group D. Most of the lumina of the follicles were devoid of colloid or in these sections where the acidophilic mass was present, vacuolations were observed.

Changes observed in the median eminence and adenohypophysis

The median eminence was observed in transverse sections of the tadpole where the optic lobes of the brain were also cut in the same plane. The most ventral part of the hypothalamus formed the median eminence and the diverticulum of the cavity of the third ventricle lay dorsal to it. The adenohypophysis was situated immediately distal to the median eminence. The thickness of the median eminence was measured by means of a graticule and the volume of the adenohypophysis measured in a manner similar to that employed for the thyroid gland; see Tables 2 & 3.

TABLE 2 — Thickness of median eminence in μ.

TADPOLE	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
1	—	10.5	36.0	49.0	52.5
2	—	10.5	28.0	42.0	56.0
3	—	10.5	35.0	49.0	56.0
4	—	10.5	35.0	42.0	52.5

TABLE 3 — Volume of adenohypophysis measured in 10^6 cu μ

TADPOLE	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
1	0.3680	1.0098	1.8477	2.5048	2.5998
2	0.3395	0.9481	1.7640	2.4034	2.6460
3	0.3528	1.0187	1.8301	2.4563	2.6063
4	0.3483	0.9349	1.8963	2.3814	2.5974

Group A

The median eminence could not be distinguished. The adenohypophysis was observed to be in contact with the floor of the infundibular recess of the third ventricle (Fig. 5). Acidophils were present in the adenohypophysis at this stage.

Group B

The median eminence was observed in these tadpoles. The most ventral part of the hypothalamus was seen to form the median eminence and the diverticulum of the cavity of the third ventricle lay dorsal to it (Fig. 6). The superior hypophyseal arteries were observed lateral to the hypothalamus external to the pia mater (Fig. 6). The adenohypophysis was seen immediately distal to this in serial sections and the median eminence, consisting of

nerve fibres, was observed as a cap of tissue superior to the adenohypophysis. The adenohypophysis consisted of cords of cells separated by sinusoids. There was an increase in size of the adenohypophysis and chromophobes and chromophils could be distinguished.

Group C

The median eminence was increased in thickness, in this group, when compared with those in Group B. However, capillaries could not be identified, with certainty, within the substance of the median eminence. The adenohypophysis was larger than in the other two groups described previously (Fig. 7).

Groups D & E.

In these animals, in the period of metamorphic climax, the median eminence had approximately doubled in thickness when compared to Groups B and C (Fig. 8). A marked feature was the presence of a large number of capillaries within the substance of the median eminence (Fig. 8). The adenohypophysis was larger and chromophobes and chromophils could be distinguished.

DISCUSSION

This study has shown that the metamorphic climax of *Rhacophorus leucomystax maculatus* is spread over a period of three days quite unlike in other anurans described by Etkin (1964). This finding is of value in evaluating the retardation of metamorphosis in aflatoxin treated animals.

The histological changes observed in the thyroid with reference to the size of the gland, follicular size, changes in the follicular epithelium, vacuolation and later evacuation of follicular colloid provide useful data as regards the activity of the gland during the later stages of metamorphosis of the animal. The decrease in volumetric size of the thyroid gland in all the specimens of Group E may be attributed to the rapid withdrawal and utilisation of colloid from the gland, by the animal, for the final stages of metamorphosis. Since all tissues were processed in a similar manner, it is unlikely that gross tissue shrinkage occurred only in specimens of animals from Group E.

Voikevich (1962) and Etkin (1963 a & b), working independently, have shown that normal thyroid activity was essential for a positive feedback effect inducing the development of the neurosecretory-median eminence system. An impairment of thyroid function could show up as a poorly developed median eminence with decreased vascularity. In this study, a corresponding increase in thickness of the median eminence was observed along with the histological changes of increased thyroid activity. There was also increase in size of the adenohypophysis.

Studies by Loeser, Mikulicz & Ritter (1955) have shown that impairment of thyroid function could be produced by specific chemical inhibitors acting on cells of the adenohypophysis concerned in the production of thyroid stimulating hormone. These workers

have shown that this produced histological changes in the thyroid gland. Since aflatoxins have been shown to cause retardation of metamorphosis, it is probable they do so by acting on the hypothalamo-adenohypophyseo-thyroid axis. Thus, it may be possible to locate the site of action of the toxin by studying the histological differences, in these three structures, in the toxin treated and control animals.

SUMMARY

Tadpoles of *Rhacophorus leucomystax maculatus* were observed during metamorphosis. The metamorphic climax in these animals lasted three days. Histological studies of the median eminence, adenohypophysis and the thyroid gland have shown that they increase in size during the later stages of metamorphosis.

EXPLANATION OF PLATES

PLATE I

FIG. 1 Transverse section of a tadpole from Group A showing single thyroid vesicle (arrow) below the fused ceratohyal and hypohyal cartilage. Delafield's haematoxylin and eosin. × 800.

FIG. 2 Transverse section of a tadpole from Group B showing paired thyroid glands (arrows). Delafield's haematoxylin and eosin. × 800.

PLATE II

FIG. 3 Transverse section of tadpole from Group C showing paired thyroid glands (arrows). Note colloid filled follicles. Delafield's haematoxylin and eosin. × 800.

FIG. 4 Transverse section of thyroid gland of tadpole from Group D showing serrated border of colloid in follicle (arrow), capillary (CAP) and empty follicle (E). Delafield's haematoxylin and eosin. × 3200.

PLATE III

FIG. 5 Transverse section of tadpole from Group A showing adenohypophysis (arrow). Note absence of median eminence in this section. Delafield's haematoxylin and eosin. × 800.

FIG. 6 Transverse section of tadpole from Group B showing adenohypophysis (arrow), median eminence (ME) and superior hypophyseal vessels (SH). Delafield's haematoxylin and eosin. × 800.

PLATE IV

FIG. 7 Transverse section of tadpole from Group C showing adenohypophysis (arrow), sinusoid (S) and median eminence (ME). Delafield's haematoxylin and eosin. × 800.

FIG. 8 Transverse section of median eminence (ME) and adenohypophysis in tadpole from Groups D & E. Note capillary (CAP) with red blood cell within it, sinusoid (S), acidophil (A) and chromophobe (CH). Delafield's haematoxylin and eosin. × 3200.

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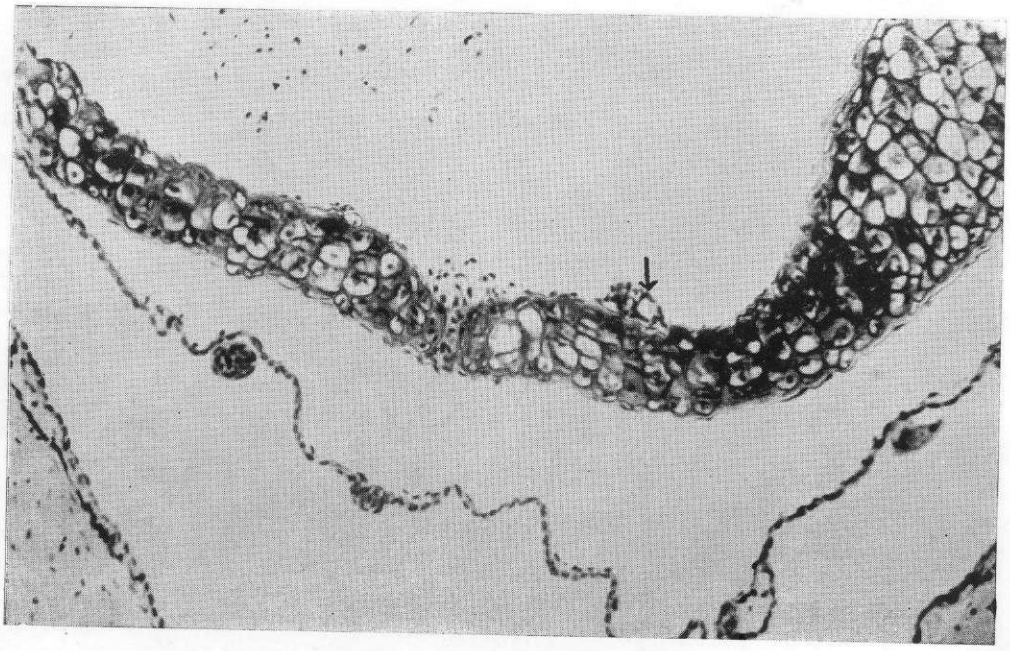

FIG. 1

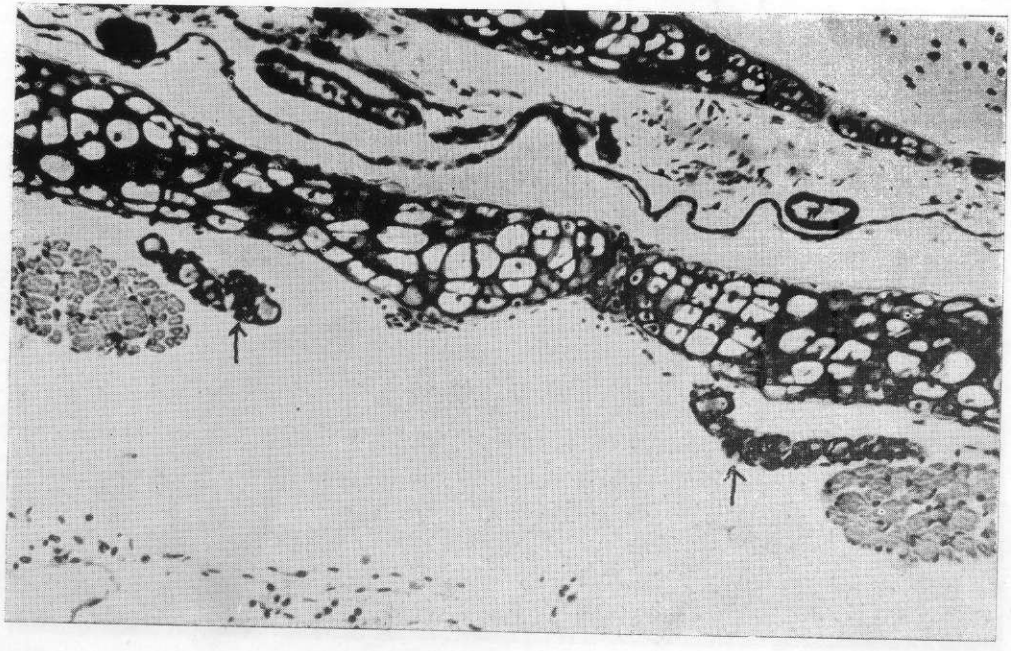

FIG. 2

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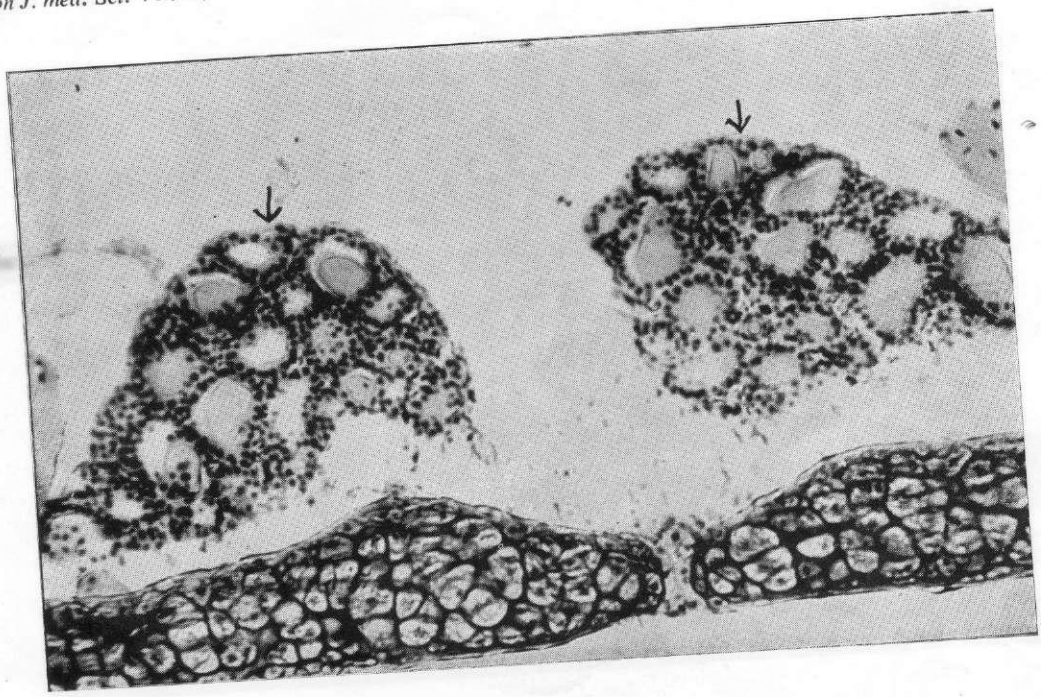

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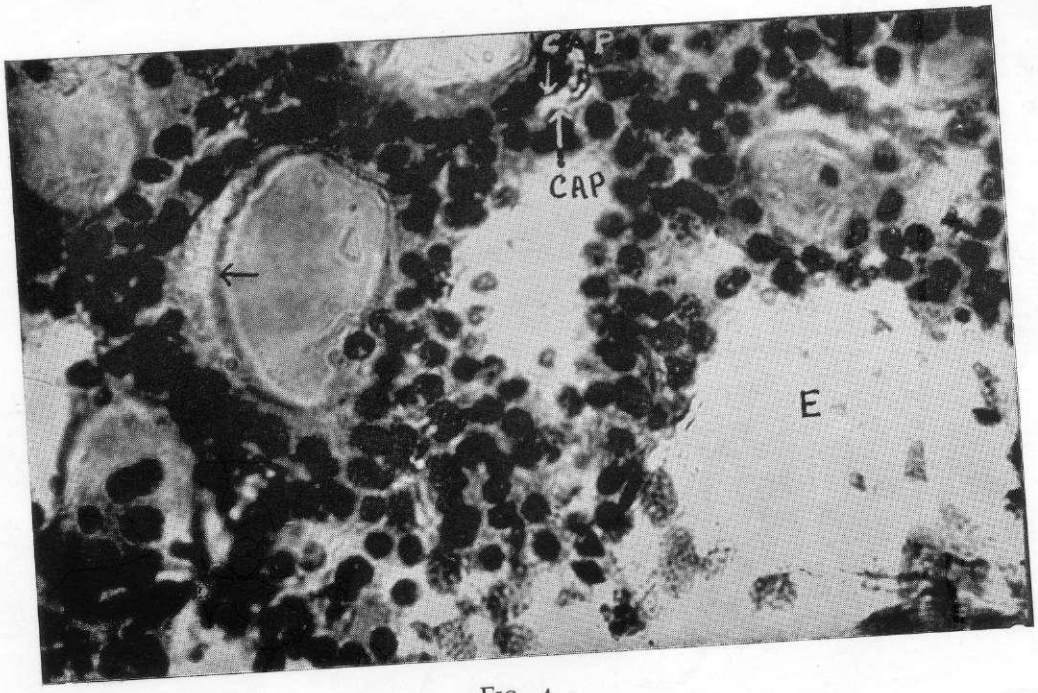

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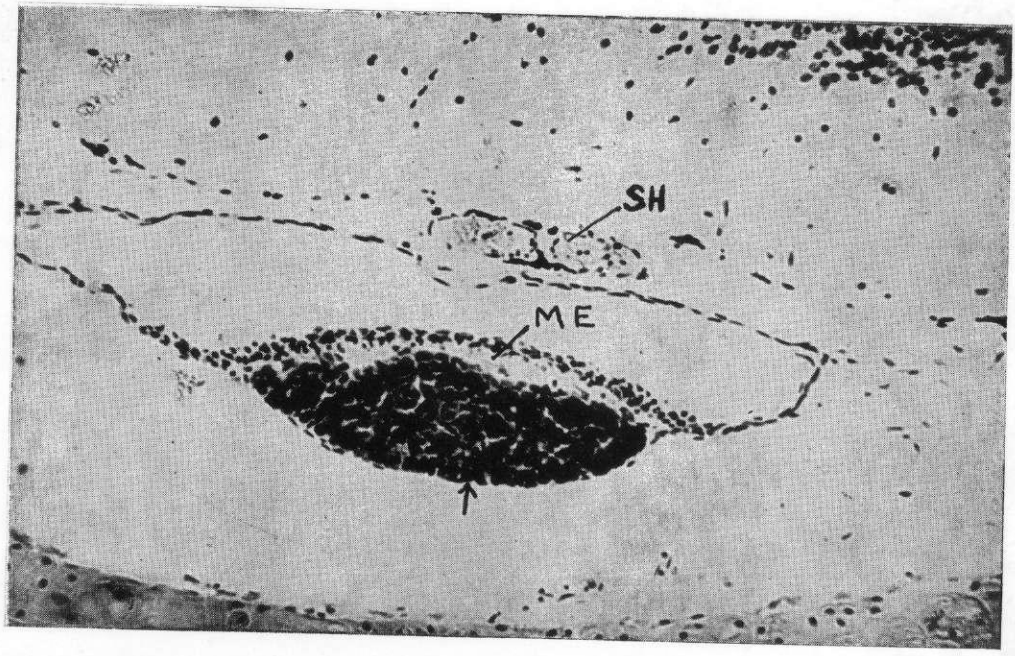

FIG. 5

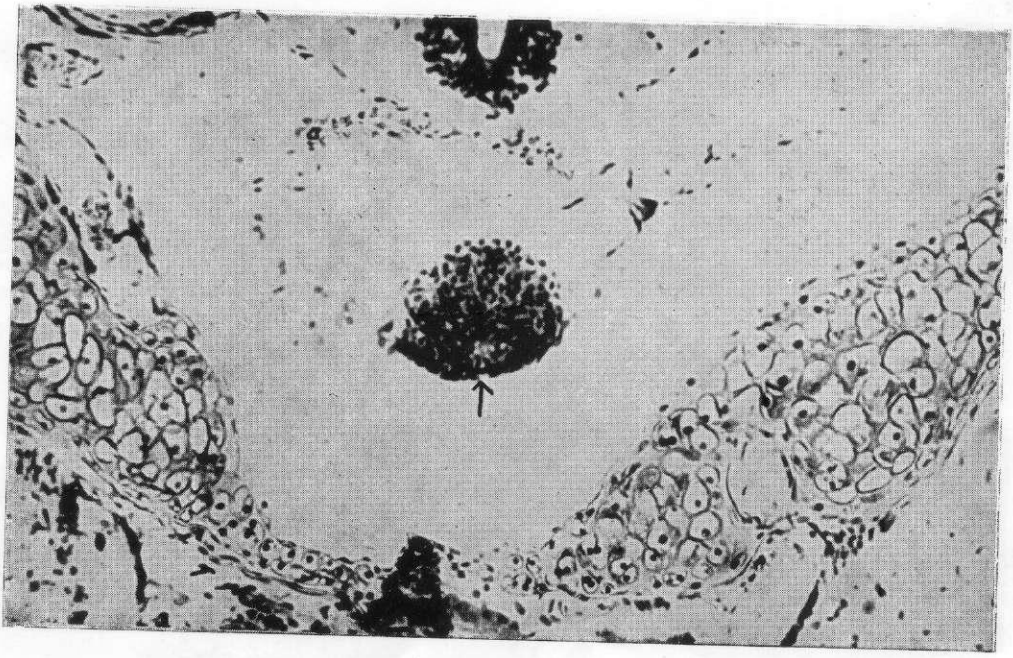

FIG. 6

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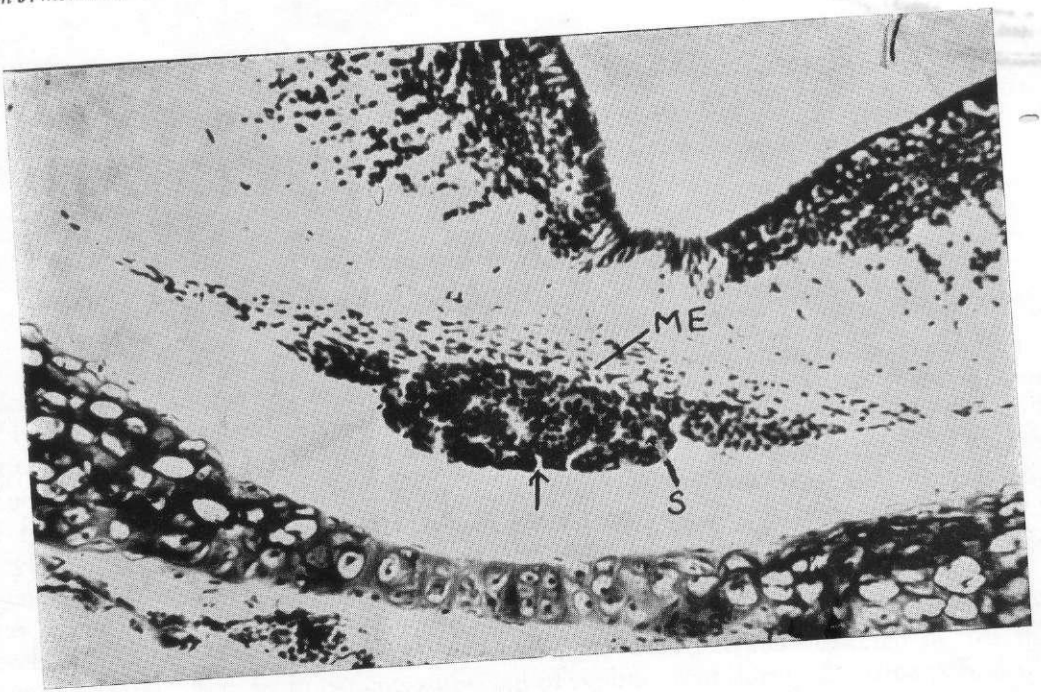

FIG. 7

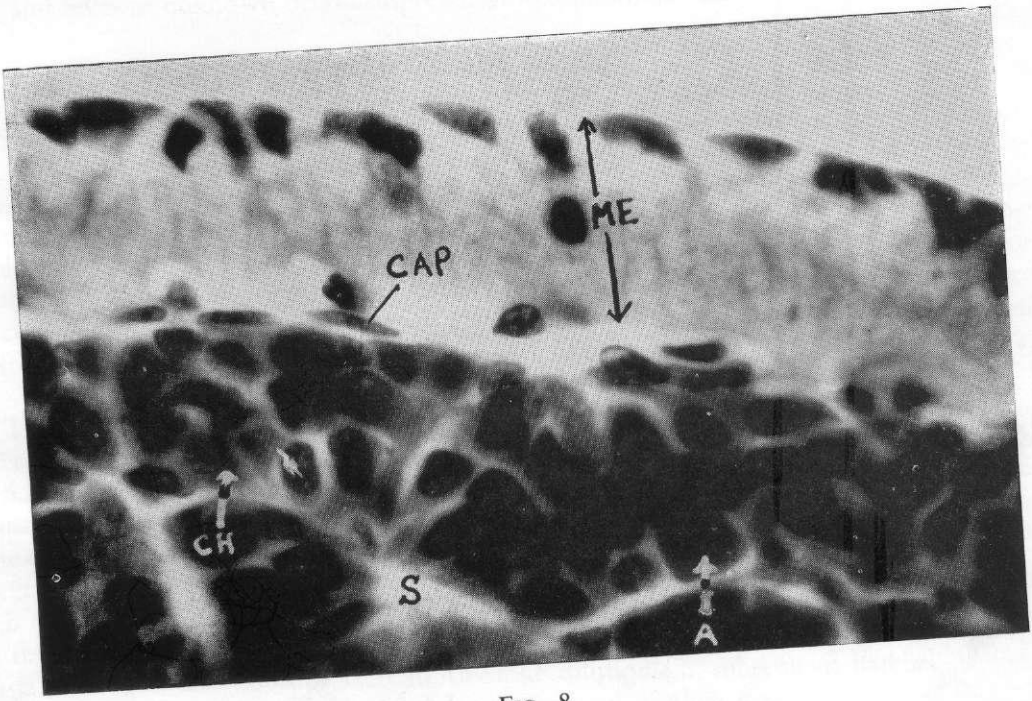

FIG. 8