ON THE STRUCTURE OF THE PITUITARY AND THYROID OF *CHANOS CHANOS* (FORSKÅL)

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INTRODUCTION.

The fry and fingerlings of the milk-fish *Chanos chanos* are known to occur in abundance in the shallow tidal creeks at several places along the coast of the Madras State usually during April to June. Although the fry and later stages are readily available along the coasts, the adults are rarely met with. In the Java Sea, the eggs of *Chanos* have been obtained beyond 30 km. from the shore (Delsman, 1929), and the fry are caught along the coast when 2-3 weeks old (Schuster, 1949). Our knowledge of the spawning habits and movements of this fish is still meagre. The rôle played by the endocrine glands in the physiological behaviour of fishes is becoming increasingly clear from many recent investigations. Among the endocrines, the pituitary and the thyroid with their various hormones originating in particular regions and during definite periods are known to regulate reproduction, growth and behaviour and hence require close examination. As physiological changes are reflected in the histology of these glands a study of these organs in the normal fish was started before attempting to find out any correlation between the changes in the glands and the physiological state of the fish.

Several accounts of the structure and function of the teleost pituitary have been published and the earlier work has been reviewed by Charipper (1937). Subsequently, Woodman (1939) described the pituitary of the Atlantic Salmon. Buchmann (1940) followed the development and the histological changes of the hypophysis during all phases in the life cycle of the herring and observed that the hypophysis has a significance to the herring similar to that in the higher vertebrates. Kerr (1940, 1942*a* and *b*), after his studies on the histogenesis and morphology of the pituitary in teleosts, gave a basic plan of the pituitary with its variations in different species in relation to their systematic position. Bretschneider and Duyvené de Wit (1947) made a careful study of the cytological changes in the pituitary of *Rhodeus amarus* in relation to its reproductive cycle and found a regular alternation of acidophils and basophils in the anterior lobe.

An outstanding contribution to the study of thyroid in teleosts was made by Gudernatsch (1911) who compared the structure of this organ in several species. Harms (1929) observed an increase in the size of the thyroid gland in the Gobies and the Blennies during their migration from aquatic to amphibious environments. Eggert (1938) in his monograph on the morphology of the thyroid dealt with the histophysiology of the organ indicating the changes in structure during different phases of activity. Hoar (1939) showed a correlation between the functional condition of the thyroid gland and the activities of the Atlantic Salmon. Later, Hoar and Bell (1950) studied the thyroid of the Pacific Salmon during various

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stages and different seasons and found heightened activity of the gland related particularly to increased metabolic work of osmoregulation in a fish prepared for life in salt water.

It is beyond the scope of this account to refer to the several experimental investigations on the endocrine physiology in fishes. The present state of our knowledge in the subject has been recently summarised by Hoar (1951).

Material and Methods.

The material included *Chanos* from 14 mm. the smallest size usually occurring along the coast, up to 550 mm. Total lengths have been given throughout in this paper. Those below 200 mm. were collected from the fry collecting centre at Chinnapalam creek and the Horseshoe Bay, Pamban, Gulf of Manaar (9° 16′ N. and 79° 13′ E.) during April-June, 1950 and 1951 and the larger ones were obtained during October, 1950 from the occasional catches by fishermen operating their nets within about five miles from the shore.

For routine preservation corrosive-formol was used and Bouin's fluid was also tried for comparison. The smaller fish were dropped in the fixative and later the heads alone were incised and left in the fixing fluid while in the larger fish the cranium was opened to expose as much of the brain as possible to ensure thorough penetration. Decalcification was avoided in most cases. Fairly successful sections were obtained after the major portion of the cranium was removed before sectioning. In materials decalcified with acids the basophilic cells of the pituitary were not properly stained. Longitudinal and transverse sections were taken at 5 or 7 micra thickness. Heidenhain's iron haematoxylin counterstained with eosin was used for general study of the histology and Mallory's aniline blue collagen stain was employed for identifying the different types of cells. The same technique was adopted for the study of thyroid also.

In describing the pituitary the nomenclature used by Kerr (1942*a*) has been followed.

Structure of the Pituitary.

14–20 mm. fry: The earlier stages of the larva have not been obtained and hence the present account starts with the study of the pituitary in the smallest size of fry that are commonly collected along the coast. At this stage the pituitary is a small elongated organ suspended from the floor of the brain and is situated behind the optic chiasma. At the place where the pituitary is connected to the brain the thalamus is thin walled. The cavity of the third ventricle does not extend into the pituitary and is lined by ependyma cells. The pituitary measures about $325 \times 70 \times 50$ micra and is held on to the floor of the cranium by means of connective tissue fibres.

The whole organ is made up of two components, the nervous and the glandular, as in all teleost pituitaries. The nervous part is continuous with the floor of the midbrain and consists of a network of fibres and a few scattered nuclei. The glandular region which covers the nervous part is separated from the latter by a thin sheet of connective tissue.

Three regions can be distinguished in the glandular part even at this stage, an anterior, middle and posterior region (Fig. 1*a*). Of these, the anterior region shows a lumen which is in connection with the buccal cavity by means of an open duct arising from the roof of the mouth just in front of the branchial arches and sloping posteriorly towards the hypophysis. This fact has already been pointed out by the author (1951). This duct or canal is lined by a nearly flat epithelium with low cells and connective tissue layer beneath.

The glandular element of the pituitary in most teleosts is known to be derived from a solid inpushing of the buccal epithelium which extends below the brain.

This hypophysial stalk usually disappears in the early stages at about the time of hatching of the embryo (Haller, 1896; Mathews, 1939; and Kerr, 1940). Buchmann (1940) while studying the hypophysis of the herring observed the presence of an open hypophysial duct ('hypophysengang') connecting the hypophysis with the buccal cavity in the early stages of the larva before metamorphosis. The structure as found in *Chanos* closely resembles that described in the herring, but the duct in the present case remains open until the fish reaches about 53 mm. long. Further observations on the disappearance of this connection in the later stages are given below.

The anterior and middle regions of the hypophysis are more or less continuous, but they can be distinguished by the difference in the nature of the cells. In the anterior region the cells are nearly columnar with about 5 micra height and oval nuclei situated in the middle. The middle region consists of a mass of round or polygonal cells 5 micra in diameter and having round nuclei. In a transverse section through this region (Fig. 1*c*) the nervous lobe is found to extend into the middle. The posterior region consists mostly of nervous tissue in the centre with a peripheral layer of cells (Fig. 1*d*) with round nuclei and their cell walls are not

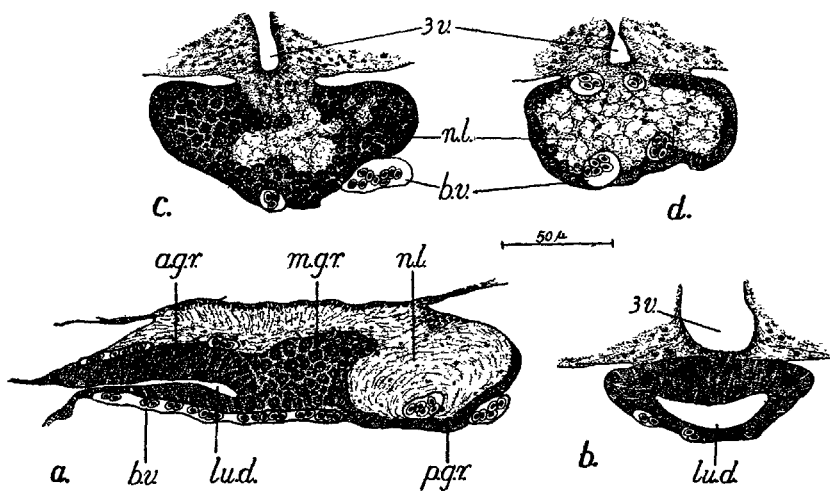


FIG. 1. *a*. Sagittal section of the pituitary of 14 mm. *Chanos*. *b*, c and *d*. Transverse sections through the anterior, middle and posterior regions respectively.

distinct. All the cells of the three different regions stain deep blue with Heidenhain's haematoxylin.

41–55 mm. stage: As the fry grows in length there is a general increase in the size of the hypophysis accompanied by significant changes. The whole organ measures about $590 \times 260 \times 160$ micra. There is great proliferation of cells in all the three regions, particularly on the dorsal side of the anterior region. As a result of multiplication of the cells the anterior region grows so as to cut off or enclose the cavity inside it. Simultaneously by further inpushings and growth of the cells more such cavities are produced. There is no regularity in the shape or size of these cavities and they are always empty. Fig. 2, *a–e* shows stages in the formation of the cavities in the anterior region. Side by side with these changes, the duct leading from the buccal cavity gets gradually constricted by about its middle resulting in the cessation of the connection between the hypophysis and the buccal cavity. The process is slow, starting when the fry grows to about 45 mm. and the connection is completely cut off when it reaches about 53 mm. in length. A vestige of this duct persists close to the anterior region of the hypophysis in the form of an

irregular space but it almost loses the cellular lining which is replaced by connective tissue. Even in later stages remnants of this space adjacent to the pituitary are noticeable.

The middle region does not show many changes. The nature of the cells remains the same but due to their growth towards the centre the small extension of the nervous lobe which was present in the early stages disappears. The posterior region continues to show a majority of the nervous tissue, but the cells in many places increase in numbers and grow in small groups towards the centre.

100–110 mm. stage: The pituitary at about this stage assumes a roughly rounded appearance and measures approximately $700 \times 500 \times 400$ micra. In a dissection of the pituitary from the ventral side of the cranium three transverse zones are distinguishable externally corresponding to the three regions of the gland. The anterior region is slightly less than half and is white in colour with meandering grooves on the surface, remotely resembling the human cerebellum. The middle

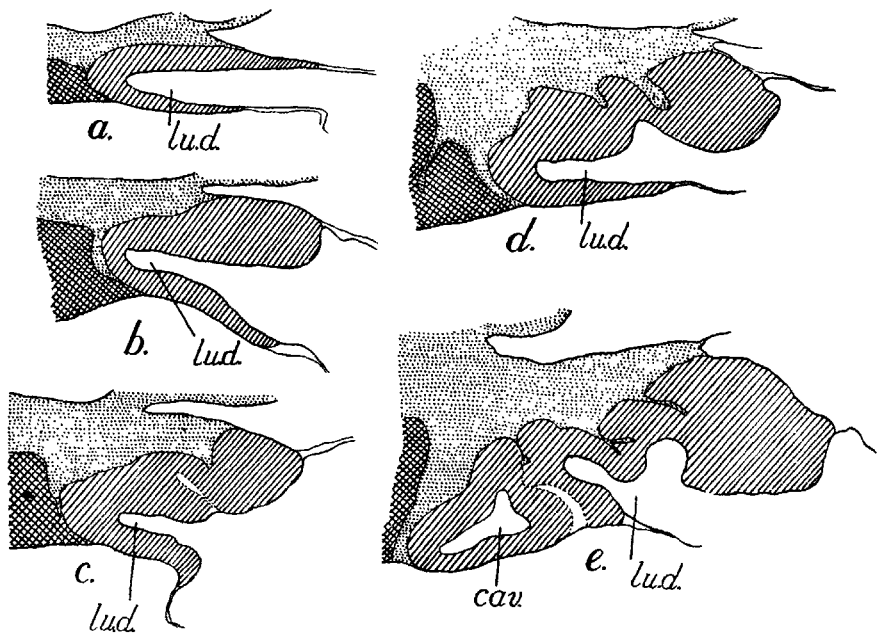


FIG. 2. *a–e*. Simplified camera lucida drawings of sagittal sections of the anterior region of the pituitary of the 14, 23, 30, 45 and 53 mm. fish showing successive stages in the formation of the cavities.

zone is very narrow and has a creamy yellow colour. The posterior zone is also as large as the anterior but it is white in colour and has a smooth surface unlike the first one. The whole gland is delicately suspended from the thalamus and is held in a depression in the floor of the cranium by strands of connective tissue.

In sections the anterior region shows an increase in the number of cavities than in the previous stage and a few in the process of formation by getting cut off from the outside. As pointed out before they have no definite size or shape. Each cavity is surrounded by a single layer of cells with an average height of 15 micra and acidophilic cytoplasm. Processes of the nervous lobe extend into the spaces among the cavities or vesicles, and between the nervous processes and cellular layer of the vesicles are seen a few blood capillaries. The middle region does not show any pronounced change. Although this region appears as a narrow one externally, towards the interior of the gland it widens to occupy roughly one-third

of the whole organ. Extensions of the nervous lobe are almost absent in this region. In the posterior region there is an increase in the cellular component and it shares the zone almost equally with the nervous part unlike in the earlier stages where the nervous lobe is greater in extent. The few cells turn out to be strongly acidophilic and exhibit a tendency to orient themselves towards the interior and adjacent to the nervous lobe.

200 mm. fish: Figure 3 shows a median sagittal section of the pituitary at this stage. It measures $1,100 \times 700 \times 500$ micra. The nervous lobe consists of an undivided network of neural fibres sloping in the form of processes towards the anterior and posterior ends of the gland. The processes of the anterior region are long and slender penetrating deeply among the vesicles while the major portion is disposed posteriorly. In the middle lobe of the gland, extensions of the nervous processes are at a minimum. The layer of collagenous or connective tissue seen between the glandular and neural component is no longer a continuous layer but a discontinuous sheet with breaks in several places. Ramifications of blood capillaries are seen in varying amounts in the different regions.

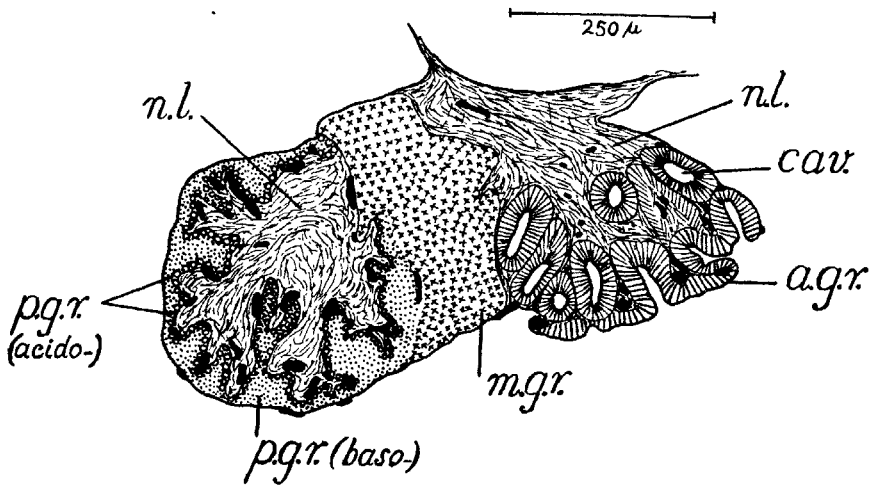


FIG. 3. Simplified camera lucida drawing of the sagittal section of the pituitary of 117 mm. fish indicating the distribution of the acidophils and basophils in the three regions.

The anterior glandular region: This is a relatively prominent region of the gland. Externally this region has an indented appearance mostly due to the presence of depressions on the surface forming vesicles. These may be large or small and irregular and are always empty. The epithelium of these vesicles consists of a single layer of cells (Pl. VII, Fig. 1) with an average height of 15 micra. The nuclei are round with a single nucleolus in each with scanty chromatin, situated near the end of the cell away from the lumen of the vesicle. All the cells are uniformly acidophilic. The cytoplasm is dense and finely granular towards the ends of the cells nearer the nervous lobe and it takes a bright red stain with acid fuchsin. Immediately close to this layer of cells is a network of capillaries surrounding the vesicles.

Cavities in the anterior lobe of the pituitary have been described in the herring by Buchmann (1940) and Kerr (1942*b*) in the trout. They have observed the presence of secretion (in the herring), or deep blue 'colloid' or detritus as in the trout *Salmo trutta* in these cavities. But such a condition has never been met with in the stages of *Chanos* and the cavities have been always found to be empty. While studying the histogenesis of the pituitary of the trout the formation of cavities in the anterior lobe of the gland has been noticed by Kerr (1940). He,

however, does not consider them as the remnants of a hypophysial cavity as seen in *Lepidosiren* (Kerr, 1933). Structurally the cavities in the pituitary of *Chanos* resemble those described in the herring and the trout, with the acidophils orientated round them, but it is not possible to compare their morphology in these different species.

The middle glandular region: This is a compact zone in the middle of the anterior and posterior regions, from which this is distinct although there is no septum or boundary separating the various regions. Unlike the condition in many other fishes, there is no mixing up of the cells with those of other regions as could be clearly marked in sagittal sections. It consists of close clusters of cells (Pl. VII, Fig. 2) without elaborate invasions of the nervous lobe processes. Capillary branchings are also relatively less in this region. The cells are small and well defined, with a diameter of 5 micra. All of them are acidophilic, staining deep brownish red with acid fuchsin or deep blue with iron haematoxylin. Thus this region differs from the corresponding lobe in the pituitary in the trout or the perch which even in early stages show acidophils, basophils and chromophobes.

The posterior glandular region: This region is characterised by the maximum invasion of the nervous lobe in the centre surrounded by the glandular cells. Unlike in the anterior lobe where the nerve processes are long and slender, in this region they are stout and blunt resulting in irregular indentations in the glandular region. An extensive branching of capillaries is also seen in the connective tissue boundary between the nervous and glandular lobes.

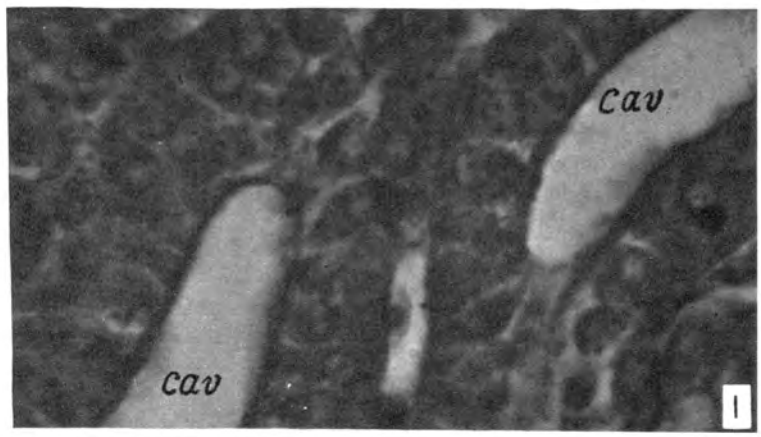
Two principal kinds of cells are found in the glandular region, the acidophils and the basophils, the former being the more common type. The distribution of the acidophils is very definite and seems to be typical. These are arranged more or less side by side regularly along the inner boundary and close to the nervous lobe and blood capillaries. In a sagittal section these appear like a continuous layer beneath which the basophils are situated. However, a few basophils may also occur among these acidophils. The acidophils are almost rounded with an average diameter of 7 micra. The chromophil substance of the cytoplasm stains brightly with acid dyes (Pl. VII, Fig. 3). The rest of the cells are dull basophils and take a light stain with basic stains. In appearance and in the arrangement of the cells this region resembles the middle lobe of the pituitary of *Perca fluviatilis* described by Kerr (1942*a*), except that in *Chanos* the extracellular acidophilic spheres mentioned by him are absent.

Structure of the Thyroid.

14–20 mm. fry: The thyroid is a small racemose gland situated near the anterior end of the ventral aorta. The number of acini or follicles varies from 8 to 12 and their size is also not very definite. In one individual the smallest follicle measures 18 micra while the largest one is 78 micra along the longest diameter and the rest are of intermediate sizes. The whole gland is supported by a framework of connective tissue which is composed of a loose fibrous network containing numerous clear spaces representing the fat vacuoles of adipose cells. A system of vascular channels traverse the connective tissue. Irregular masses of pigment of yellowish brown colour are often seen embedded in the connective tissue of this region.

The follicles are lined by a single layer of epithelium and many of the follicles are connected together. There is no distinct basement membrane for the epithelium. The cells are nearly cuboidal and the cell walls are distinct. They have an average height of 7 micra and the nuclei are rounded, 3-4 micra in diameter with two prominent nucleoli in each nucleus. The cytoplasm is scanty and faintly vacuolated in appearance, particularly towards the inner ends of the cells.

The follicles have colloid within them which stains deeply with iron haematoxylin towards the periphery but lightly towards the centre. The colloid shows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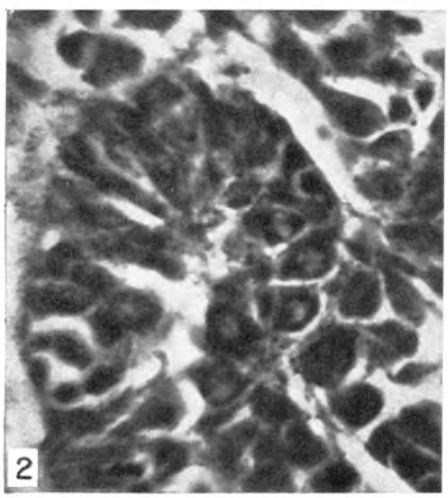
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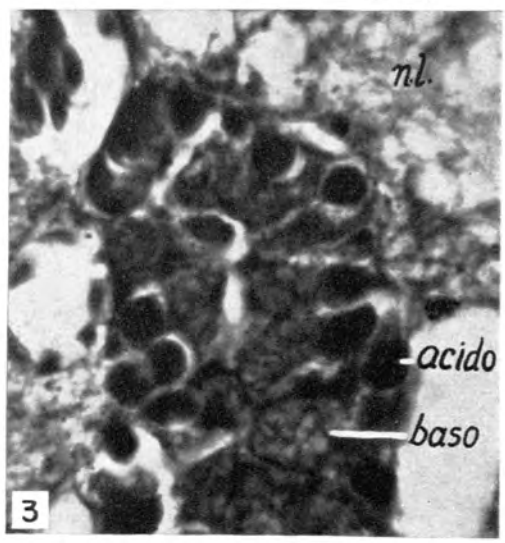
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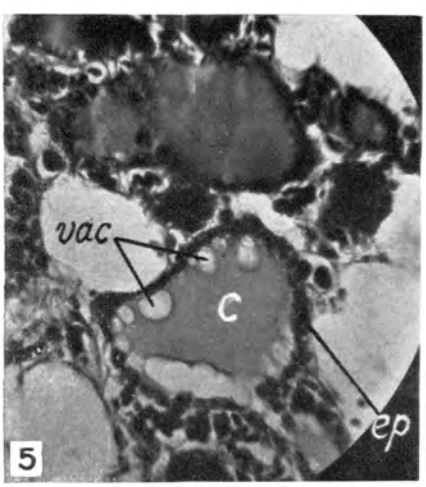
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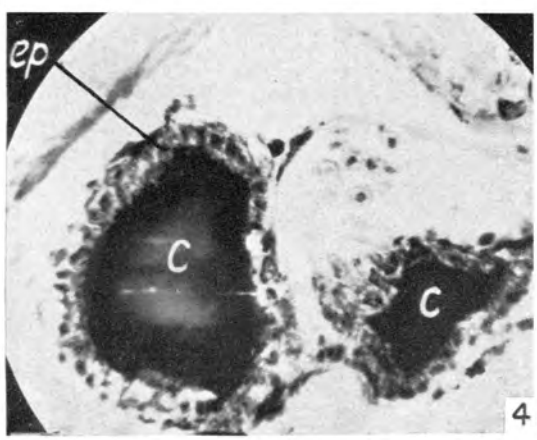
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specific affinity for acid stains. No vacuoles are seen in the colloid and the whole appearance of the gland suggests that it is in a quiescent state (Pl. VII. Fig. 4).

Groups of undifferentiated glandular cells without the formation of the colloid or any cavity in the centre are often met with in the follicles. These suggest that the later follicles are formed by proliferation of cells from the epithelium of older follicles as has been observed in the Atlantic Salmon (Hoar, 1939).

The histological condition of the thyroid in the 20 mm. fry is more or less similar to that seen in the 14 mm. fry. The number of follicles has increased and they have extended to the more posterior regions round the ventral aorta. The nature of the epithelial cells of the follicles remains unchanged, consisting of cuboidal cells with round nuclei, situated in the middle of the cells. Colloid is prominently observed in the follicles.

20–30 mm. fry: The gland has grown diffuse by the multiplication of the number of collicles whose size as well as shape vary; the largest one measuring 52 micra in diameter. The acidophilic colloid fills the follicles and the epithelial cells are cuboidal and without vacuoles, having an average height of 6 micra.

40–60 mm. fingerling: The glandular tissue has enlarged considerably spreading to the posterior regions with numerous follicles, the largest with a diameter of 60 micra. Acidophilic colloid is present in all the follicles. The cells of the epithelium are almost the same as in the previous stages. In one preparation, however, some of the cells showed one or two darkly staining large granules showing the same staining properties as the central colloid. Whether these represent newly formed colloidal material within the cells before it has moved to the lumen of the follicle or they are merely artefacts caused within the cells while preserving cannot be said at present.

100–120 mm. fish: Compared with the previous stages there is an increase in the number of follicles, most pronounced anteriorly and in the dorso-lateral portions of the ventral aorta. While the follicles are more closely packed in the anterior region, they are scattered posteriorly. A few are present in the ventral regions but they never extend to the caudal regions of the ventral aorta. In the regions where the follicles are crowded there is an engorgement of blood in the interfollicular spaces. The undifferentiated prefollicular tissue is seldom evident and histologically the thyroid acini are well separated. The follicles are nearly spherical but they vary much in size, the largest one encountered being 100 micra in diameter.

The epithelium is still cuboidal with cells of 4-5 micra in height and round nuclei occupying the middle of the cells. The colloid mass which completely fills the follicles is acidophilic in nature.

270 mm. fish: Here again there is a considerable increase in the bulk of the gland and an enlargement of the follicles. Fig. 4 shows the distribution of thyroid

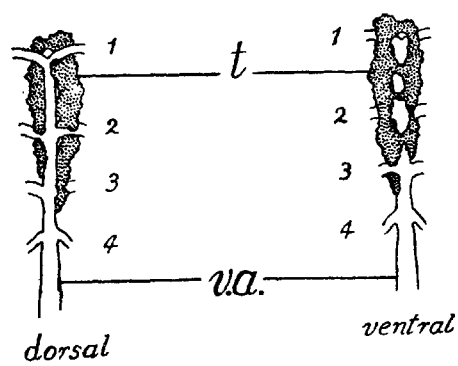

FIG. 4. The distribution of thyroid around the ventral aorta in a 240 mm. fish. 1–4 represent the branchial arches. ×3.

material in a fish of 240 mm. length. Side by side with the development of the glandular component, there is great increase in the vascularization of the gland. The maximum size of the follicles at this stage is 110 micra. Many of the follicles have acidophilic colloid filling them while some of them are seen with large vacuoles in the periphery of the colloid (Pl. VII, Fig. 5). The epithelium is made up of cuboidal cells 4 micra in height with scanty cytoplasm and round nuclei.

550 mm. fish: This is the largest sized fish whose thyroid has been studied. Due to the enormous increase in the thyroid material and the well developed interfollicular vascularization, the whole gland appears to be more organized as compared with the dispersion of the follicles of the earlier stages. The epithelial cells are always in close contact with the blood system of the interfollicular spaces.

The follicles are fully distended in most cases with colloid. They are thus considerably larger than those met with in the smaller fish. Follicles with a diameter of 150 micra are common at this stage. The epithelium, which may be described as squamous, consists of low cuboidal cells with an average height of 3 micra. The nuclei are slightly oval. The colloid does not seem to show the same degree of affinity to acid stains.

General Considerations.

The beginnings of histological differentiation in the pituitary at an early stage and the presence of colloid in the thyroid vesicles in the youngest fry captured along the coast would probably indicate that these glands have become functional in them. The early appearance of the acidophils of the anterior and posterior lobes is often associated with the general metabolism of the body, including growth (Kerr, 1939), while the appearance of the basophils in the middle lobe suggests a possible association of these cells with the maturation of gonads (Matthews, 1939). All the specimens examined for this study were juveniles in which the sexes could not be distinguished and this may explain the absence of basophils in the middle lobe in any of the stages described.

For distinguishing the various types of cells, the technique adopted by Kerr (1942*a*) has been followed here. The use of the terms 'acidophils' and 'basophils' has its limitations in view of the conflicting results obtained by different workers as regards the staining properties of the cells and cytoplasmic inclusions in the herring pituitary which were stained blue with Delafield's haematoxylin and red with Azan were basophils as opposed to the usual interpretation. A similar comparison between adjacent sections of *Fundulus* pituitary has been made by Grace E. Pickford (personal communication of unpublished observations) who has also come to the same conclusion.

Factors like food, season, condition of gonads and changes in the salinity of the environment are said to be responsible for variations in the histology of the thyroid. The *Chanos* larvae and young ones up to 200 mm. were collected from their natural surroundings in the shallow coastal regions during the summer months where the water had a salinity of 34‰ and the temperature varied from 25°C. to 32°C. in the course of twenty-four hours. The larger fish were caught within five miles from the shore where the salinity was about 34·5‰. All the stages of the fish examined here showed the thyroid follicles to be uniformly rounded with a low epithelium of nearly cuboidal cells and filled with acidophilic colloid, thereby suggesting a resting condition. Due to the budding of the follicles and enlargement in their size, the bulk of the thyroid material increases and grows more around the caudal regions of the ventral aorta. The colloid is formed even in the larvae and is retained within the follicle throughout. Withdrawal of the colloid has not been observed except in a single instance of a 270 mm. fish but the structure of the gland in the larger fish did not suggest having undergone any involution. In the metamorphosing herring Buchmann (1940) observed a great increase in the height

of the thyroid epithelium and presence of vacuoles in the chromophilic colloid. Pronounced histological changes were not noticed in *Chanos* larvae of 14–20 mm. size. Similarly no significant changes in the gland were seen in any of the later stages. Perhaps the changes in the environment are not very marked to alter the state of the gland. These points, however, remain for future study.

Summary.

A histological account of the pituitary and thyroid of the early stages of *Chanos chanos* is given.

A connection between the buccal cavity and the anterior region of the pituitary is observed in fish up to 55 mm. length. Changes in the nature of the cells in the anterior, middle and posterior glandular regions take place gradually so that in the later stages the anterior and middle lobes consist of acidophilic cells while both acido- and basophils are present in the posterior lobe.

The thyroid increases in bulk as the fish grows, but under normal environmental conditions the gland does not show any marked degree of activity.

Acknowledgements.

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Explanation of Photomicrographs.

Plate VII.

Fig. 1. Anterior glandular region of the pituitary of a 200 mm. *Chanos* showing the acidophils arranged round the cavities. ×500.
2. Middle glandular region with only acidophils. ×500.
3. Posterior glandular region showing the acidophils orientated along the boundary near the nervous lobe. ×500.
4. Thyroid follicles in a 14 mm. fry. ×500.
5. Thyroid in a 270 mm. fish, with vacuoles in some of the follicles. ×250.

Key to Lettering.

acido .. Acidophils.
a.g.r. .. Anterior glandular region.
b.v. .. Blood vessel.
baso .. Basophils.
c. .. Colloid.
cav. .. Cavity of the anterior region.
ep. .. Epithelium of the thyroid follicle.
lu.d. .. Lumen in continuation with the hypophysial duct.
m.g.r. .. Middle glandular region.
n.l. .. Nervous lobe.
v.a. .. Ventral aorta.
vac. .. Vacuoles.
3v. .. 3rd Ventricle.

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