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THE DEVELOPMENT OF THYROID IN AN ELASMOBRANCH *CHILOSCYLLIUM INDICUM* (GMELIN)

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ABSTRACT

The development of thyroid in the elasmobranch *Chiloscyllium indicum* was found to be markedly different from that had been described in other elasmobranchs. It developed as a mid-ventral groove on the floor of the pharynx and the cells were considered to be endostylar in origin. The development of the gland as observed in embryos measuring between 1.9 mm and 16.8 mm long was described, compared and discussed with those in other elasmobranchs.

INTRODUCTION

STUDIES on the thyroid gland in cold blooded vertebrates (Lynn and Wachowsky, 1951) show that the origin of the thyroid anlage differs in the various groups of fishes and cyclostomes. The gland may be formed either hollow or solid from the pharynx as a diverticulam or as an outpushing from its ventral wall. In Lamprey *Bdellostoma* (Stockard, 1906), the thyroid originates as a longitudinal groove in the pharyngeal floor extending over the whole gill area; but according to Norris (1918) in the elasmobranch *Squalus acanthias*, it arises as a solid epithelial bud. Generally, in teleosts the thyroid originates as a hollow evagination of the pharyngeal floor (Gudernatasch, 1911), but in Salmon, Hoar (1939) has shown the thyroid originating as a solid knob of cells. It is thus seen that the nature of the thyroid anlage differs in the various groups of fishes.

The Indian region is rich in species of Elasmobranchs, but owing to their peculiar habits of reproduction, very few attempts have been made to study the development of the Indian species. The available references are only life history studies and morphological descriptions of the embryos of a few sharks by Alcock (1890, 1892), Woodmason and Alcock (1891, 1892), Southwell (1910), Southwell and Prashad (1919), Smedley (1926, 1927), Deraniyagala (1934, 1945), Aiyar and Nalini (1938), Setna and Sarangdhar (1949, 1950) and Setna (1949).

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MATERIAL AND METHODS

The breeding season of *Chiloscyllium indicum* lasts from December to March. Rearing of sharks in the aquarium tanks for procuring eggs was not very successful.

Therefore gravid female sharks (*C. indicum*) were collected from the fishing boats at Cochin during the egg laying season. These sharks contain 2–4 egg cases. The egg cases were removed carefully, after opening the fish and were then kept in a bottle of sea water. The egg cases were then numbered with, on small bits of plastic sheets stuck on the adhesive filaments of the egg cases. The egg cases were then transferred to a perforated plastic container and kept submerged in the sea water at the harbour. The egg cases were opened at intervals of one day or less in the case of early stages and fixed with Bouin's fluid or Smith's fluid. Ordinary paraffin embedding method was used and the serial sections were cut at 5μ thickness. Heidenheins iron haematoxylin was found to be the best nuclear stain. Delafield's haematoxylin and Eosin were used for the later stages.

Development of the Thyroid Rudiment

In a 1.9 mm long embryo of *C. indicum* the arched endoderm layer closes to form a gut rudiment about 350 μ long in the anterior region. This is the pharyngeal region of the gut. A median groove, approximately 60 μ long appears on the floor of the pharynx, extending backwards from the posterior end of the second branchial arch. This groove is rather shallow at its posterior end, but it becomes progressively deeper and wider towards its anterior end. The appearance of this groove marks the first stage in the development of the thyroid rudiment. With the formation of the groove the median ventral wall of the pharynx is pushed outwards as a longitudinal ridge, the groove (PL. I A, gr) extending into it as a deep narrow slit. In other words the thyroid rudiment is formed as a median longitudinal outpushing of the floor of the pharynx (PL. I A, th.r). The side walls of the ridge are formed by a continuation of the endodermal wall of the pharynx. But the cells are elongated and columnar, so that the ridge appears as a deep keel with bulging lateral sides. Posteriorly the groove is comparatively shallow. Here also the sides of the ridge are formed of a single row of columnar cells; but along its ventral side the cells proliferate rapidly and become a thick cluster with the result that this region becomes prominently thick and rounded. In the anterior part of the ridge all the cells lining the groove are uniformly columnar with oval nuclei. But in the posterior half, although the cells forming the side walls of the ridge are similar to those in the anterior part of the groove, along its ventral side, cell limits are not discernible and the nuclei which are more closely crowded together are smaller and spherical. Moreover, cells budded off from the peripheral epithelium move towards the centre, thus obliterating the pharyngeal groove in the ridge (PL. I B). So in this region the ridge is almost solid.

By the time the embryo is 3.8 mm long, the thyroid rudiment reaches a length of about 70μ. The caudal part of the ridge is more conspicuously circular in cross section, and it is attached to the floor of the pharynx by a narrow isthmus. In other words, the line of attachment of the rudiment to the floor of the pharynx is deeply constricted. The isthmus-like part and the outer wall of the rudiment is lined by a single layer of oval nuclei (p.N.) which are larger than those of the pharyngeal endoderm. The space inside also contains a few scattered nuclei similar to those forming the outer wall and a number of small deeply staining pigment granules (Fig. 1 f, pi. f). While these changes have taken place in the caudal half of the rudiment, the anterior part is still unchanged. Here the pharyngeal groove extends into the ridge as a deep vertical slit (PL. I C); and the wall of the ridge is formed of a single row of large nuclei without cell limits. With the further growth of the embryo, the thyroid rudiment also increases in length. Its posterior half becomes more and more constricted, and the constriction slowly advances towards the cephalic end of the rudiment. In an embryo 5.1 mm long, the isthmus-like part connecting the rudiment with the floor of the pharynx is very narrow. The hinder most part of the rudiment is now fairly thick and cylindrical. But, towards the cephalic end

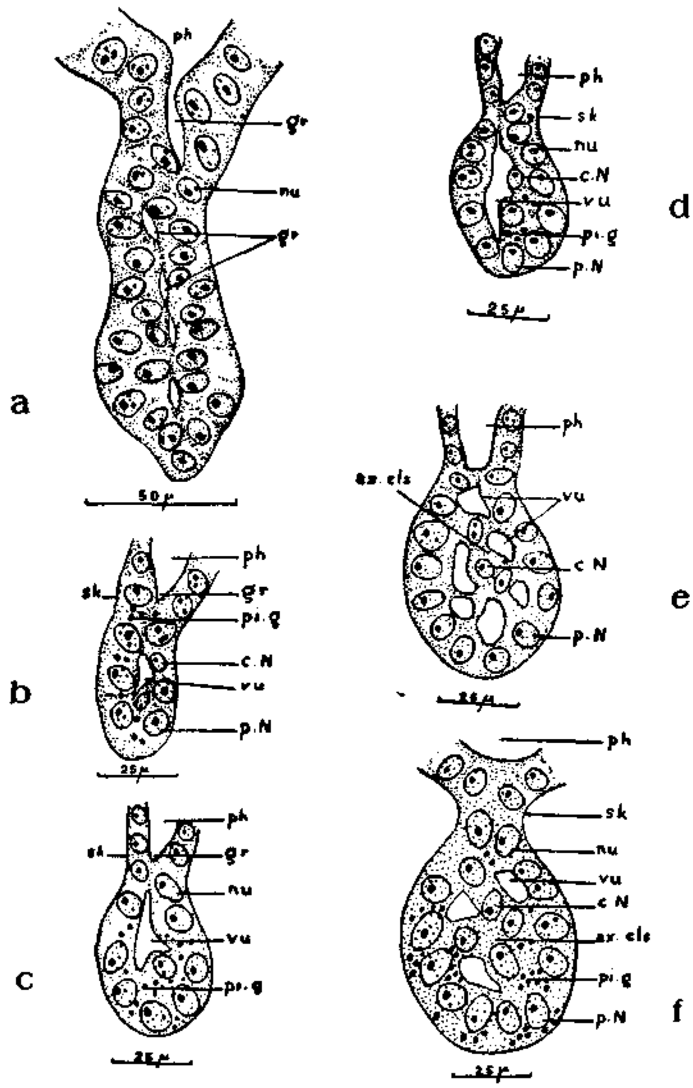

Fig. 1 a. T. S. of thyroid in a 5.1 mm embryo, b - e. T. S. through the various regions of the thyroid in a 13.0 mm embryo; and f. T. S. through the attached region of the thyroid in a 3.8 mm embryo.

it is keel-shaped. Here it is still lined by a single layer of large oval nuclei, and the protoplasm surrounding these nuclei extends inwards from the two sides, thus almost obliterating the pharyngeal groove, which formerly extended into it (Fig. 1 a). However, it will be noticed that the ventral tip of the groove still persists as a small lumen, surrounded by the inner boundary of the peripheral protoplasm.

When the embryo is 5.4 mm long, the hind part of the thyroid rudiment upto a length of about 18 μ is detached from the floor of the pharynx. As the embryo increases further in length this process of separation extends forwards and in an embryo about 13.0 mm long, in which the thyroid rudiment is 120μ long, only the anterior 30μ of the rudiment still retains its connection with the pharynx. At this stage the thyroid rudiment appears as a solid cylindrical rod lying beneath the floor of the pharynx and connected with the latter only by a narrow stalk at its anterior end. The detached part of the rudiment is circular in cross sections (PL. I D). No cell boundaries are visible; but the large spherical nuclei are arranged in a single peripheral row (p.N) and the peripheral protoplasm is also restricted in such a manner as to leave a central space, which is filled with an axial cluster of nucleated protoplasm (ax. cls), between which and the peripheral protoplasm there is a wide ring-like space bridged at intervals by protoplasmic strands. The presence of these radiating protoplasmic strands subdivide the ring-like space into a number of irregular cavities. The pigment granules, which were fairly abundant inside the rudiment during the early stages, have now completely disappeared from the detached part of the rudiment. However, a few scattered granules are noticed in the stalk connecting the rudiment with the floor of the pharynx.

Cross sections passing through this stalk (Fig. 1 b, sk) show that at the extreme anterior end, the stalk is more or less keel-shaped and without any constriction between it and the floor of the pharynx. It is almost solid with only a very narrow median lumen (vu), immediately below the floor of the pharynx. Most of the nuclei are peripherally arranged (p.N), but there are also a few smaller nuclei in the central part (c.N). The next section (Fig. 1 c) shows the beginning of a constriction between the stalk and the floor of the pharynx. The median lumen is more conspicuous and a number of pigment granules (pi.g) are noticed on the sides and beneath the lumen. In the third section (Fig. 1 d) the constriction is more conspicuous; the nuclei are more regularly arranged along the periphery and the lumen is much larger and it appears as a vertical laterally compressed space slightly displaced towards one side. There are a few internal nuclei between the periphery and the lumen. In the next section (Fig. 1 e) the lumen is broken up into six irregular spaces and the internal nuclei now occupy a position in the axial part of the rudiment. At the hind end of the stalk the constriction between it and the floor of the pharynx is quite deep. The thyroid rudiment, although still attached to the floor of the pharynx, is distinctly demarcated from it by a line ot separation. The lumen between the central cluster of nuclei and the peripheral row is fairly wide and broken up into small irregular vacuole-like spaces by protoplasmic strands (PL. I D).

In an embryo 16.8 mm long, the stalk connecting the thyroid-rudiment with the floor of the pharynx has completely disappeared and the thyroid rudiment appears as an elongated cylindrical rod, extending backwards to a length of 156μ and terminating blindly in front of the heart rudiment. The structure of the thyroid rudiment at this stage is the same as in the earlier stage; but the vacuole-like spaces are much more conspicuous, and the axial cluster of nuclei more dense. However, the pigment granules which were present in the previous stages have now completely disappeared so that the rod-like thyroid rudiment now consists of a peripheral row of closely arranged large oval nuclei and an axial cluster of smaller but spherical nuclei. As already stated these axial cells, eventhough slightly different in their appearance from the peripheral cells, are no doubt derived from the latter by cell division.

It may be interesting to point out that the pigment granules are present only in the region were the thyroid rudiment is attached to the pharynx. These granules progressively disappear as the line of separation extends further and further forwards, so that in the 13.0 mm long embryo the granules are present only in the stalk, from where also they disappear as soon as the rudiment in separated from the floor of the pharynx.

Discussion

From the above description of the development of the thyroid rudiment in *C. indicum*, it will be seen that it differs markedly from what has been described in other elasmobranchs so far studied. It is generally believed that the thyroid gland in vertebrats, including fishes, originates as a mid-ventral outpocketing of the anterior pharyngeal floor (Nelsen, 1953). But despite this generalisation regarding its origin, it has been found, that in elasmodranchs the thyroid originates differently in the few species which have so far been studied, such as *Squalus acanthias* (Müller, 1871; Norris, 1918), *Scyllium* (Balfour, 1878) and *Chlamydoselachus* (Goodey, 1910). The earliest account of the thyroid of an elasmobranch is that of Müller (1871). He recorded that in *S. acanthias* the thyroid possesses a persistent thyroglossal duct. Goodey also found in the thyroid of *Chlamydoselachus*, a condition similar to that noted by Müller (1871). Later Balfour (1878) who described the development of thyroid in *Scyllium* embryos observed that this gland appears as a diverticulum from the ventral surface of the throat. In front, it contains a groove which opens into the throat, while posteriorly, the diverticulum is a solid rod. This solidification is effected by the columnar cells which line the groove, extending and meeting in the centre. Soon after, the lumen is thus obliterated and small cells make their appearance in the interior of the thyroid body, probably budded off from the original columnar cells.

The findings of Müller were, however, contradicted by Norris (1918), who reinvestigated the development of the thyroid in *S. acanthias*. According to him the rudiment of the thyroid gland makes its appearance in embryos approximately 4.0 mm long, as a solid localised thickening of the endodermal lining of the floor of the pharynx. He observes that no groove or pouch is present in the floor of the pharynx at the point where the thyroid first appears. The distal end of the bud increases in length and extends backwards attached to the pharynx by a peduncle-like narrow neck. By the time the embryo is about 19.0 mm long, the rudiment is completely cut off from the pharynx and it appears as a columnar structure with rounded ends and avoid cross sections.

A completely different account is presented by Melouk (1949) regarding the origin of the thyroid gland in *Rhynchobatus djiddensis*. In this species, according to him, when the embryo is 5.0 mm long the thyroid gland anlage is formed behind the mouth in the fleshy basal portion, formed by the ventral connection between the mandibular arches. He asserts that it is not a ventral longitudinal groove of the anterior pharynx as might be expected, but simply a slightly evaginated compact thickening of the anterior most part of its bottom, intimately related to the mandibular arches and the mouth, and that any relation with the posterior part of the pharynx is to be considered as secondary. Although Melouk (1949 b) maintains that the origin of the thyroid in *R. djiddensis* represents the primary character of the gland, it conflicts with the earlier observations, both in regard to the place of origin and subsequent development. However, it is possible that the fleshy basal portion between the ventral connections between the mandibular arches, may actually represent the mid-ventral outgrowth of the pharyngeal floor as in other vertebrates. But even with such an interpretation the place of origin between the mandibular arches is difficult to reconcile, with the generally accepted view, as stated by Kerr (1919), that it arises at the level of the hyoid.

The development and origin of thyroid in *S. acanthias* is more or less similar to the development of this gland in higher vertebrates. On the other hand, the process of development observed earlier by Müller (1871), Balfour (1878) and Goodey (1910), envisages a somewhat primitive condition, which is reminiscent of the evolutionary history of the gland as postulated by Müller (1871). Based on the presence of the thyroglossal duct in the thyroid of *S. acanthias*, Müller (1871) advanced the view that the thyroid may be regarded as a homologue of the endostyle of *Tunicates* and *Amphioxus*. This correlation between the thyroid and the pharynx appears to be slightly more emphasised in *C. indicum*, in which during the early embryonic development the pharyngeal groove appears as a median-ventral longitudinal outpushing of the pharyngeal cavity along the entire length of the gill region. It arises not as a diverticulum from the ventral surface of the throat, but as a median groove on the floor of the pharynx as in the Lamprey *Bdellostoma*.

In *Bdellostoma* (Stockard, 1906) the thyroid originates as a groove in the pharyngeal floor and extends through the whole gill area. Although Stockard (1906) does not consider this condition as evidence of any close phyletic relation to the endostyle of *Amphioxus*, Leach (1939) concludes on the basis of later studies on *ammocoete* larva that the cells of the thyroid are quite clearly derived from certain elements of the endostylar region of *ammocoete* larva. Since the origin of thyroid in *C. indicum* is almost identical with that of *Bdellostoma*, it may be concluded that in *C. indicum* also, the cells of the thyroid may be endostylar in origin, in view of its development as a mid-ventral groove on the floor of the pharynx. Later, the cavity of this groove is obliterated by the inward elongation of the peripheral cells and the multiplication of these cells by budding. However, a perusal of PL. I B which represents a section of the posterior end of the rudiment of a 1.9 mm long embryo, shows that the lateral walls of the rudiment meet in the centre. Although this process results in the obliteration of the greater part of the pharyngeal groove, its ventral end persists as a narrow median space. A similar condition is also noticed in the anterior region of the rudiment in a 5.1 mm long embryo (Fig. 1 a) and also in the stalk of the 13.0 mm long embryo (Fig. 1 b). The mode of origin of this gland, its structure and relation with the phrynx, in the early embryonic stage, resemble the endostyle in the ammocoete larva of *Petromyzon* and just as the endostyle of *ammocoete* transforms into the thyroid of the adult, so also in *C. indicum*, the ventral longitudinal pharyngeal groove of the embryo transforms into thyroid during later development. It will thus be seen, that the development of thyroid in *C. indicum* provides a good example of the evolution of the thyroid from the primitive endostyle.

In *S. acanthias* according to Norris (1918) the gland is solid upto the time of its separation from the pharynx and cavities developed in it have no relation with any early duct or pouch. According to him, completely closed cavities are secondary formations, appearing in the thicker parts of the gland only after it has severed its connection with the pharynx. Müller described a lumen in the thyroid rudiment of *S. acanthias*, but Norris (1918) believes that this lumen corresponds with the cavities observed by him in his embryos. He suggests that they may have been formed in response to a tendency to produce 'the ancient lumen or duct of the ancestral gland', but whatever may be the origin and nature of these cavities in *S. acanthias*, there is no doubt that in *C. indicum* the cavities found in the thyroid rudiment are derived directly from the pharyngeal cavity.

In other details the structure of the thyroid rudiment in *C. indicum* agrees closely with that of *S. acanthias*. Differences in the size of the cells between those along the periphery and the centre were noticed by Norris (1918) in *S. acanthias* and also by Balfour in *Scyllium* embryos. But no attempt had been made so far to offer any suggestion regarding the significance of this difference.

Another interesting point observed by Norris (1918) in *S. acanthias* which has also been noticed in *C. indicum*, is the presence of pigment granules. Norris (1918) believes that a large amount of pigments is found in the parenchyma of the gland in the embryos of *S. acanthias* and that these pigment granules increase in amount upto the time of the separation of the gland from the pharynx, their maximum concentration being at the point of separation and that, thereafter they disappear rapidly. In *C. indicum* also, similar granules have been observed. During the early stages the pigment granules are present in the entire length of attachment of the rudiment to the pharynx. As the line of attachment recedes towards the anterior end, the pigment granules progressively disappear from the detached part ot the rudiment. Ultimately when the rudiment is connected with the pharynx only by a narrow stalk, a few pigment granules are noticed only in the stalk.

Similar pigment granules have been noticed by Balfour (1878) also, but no attempt has been made to suggest any function for them, except that they may be associated with the division or separation of the rudiment from the floor of the pharynx.

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PLATE I. A, B. T. S. of the pharynx in 1.9 mm embryo (Microphotograph x 333 and x 285); C. T. S. of thyroid in 3.8 mm embryo (Microphotograph x 255); and D. T. S. of thyroid in 11.4 mm embryo (Microphotograph x 240). (ax. cls = axial cluster of cells, br. a = branchial arch, br. p = branchial pouch, c. N = central nucleus, gr = groove, nu = nucleus, ph = pharynx, pi. g = pigment granules, p. N = peripheral nucleus, sk = stalk, st. = stomach, th. r = thyroid rudiment and vu = vacuole).

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