INTRODUCTION

Since the publication of Balfour's classical monograph (1878) on the development of Elasmobranchs, a number of important papers on this subject has appeared. Of these, the most noteworthy are the works of Parker (1879), Marshall (1881), van Wijhe (1882, 1904, 1915, 1922 & 1923), Dohrn (1884, 1886), Bengtson (1885), Beard (1889-90), Platt (1891), Frolich (1891, 1892, 1901), Rabl (1892, 1897), Ziegler (1892 & 1908), Sedgwick (1892), Peabody (1896), Kopsch (1897, 1931), Braus (1899, 1906, 1914), Rückert (1899), Dean (1902), Gaupp (1906), Brehm (1909), Goodrich (1918, 1930), de Beer (1922, 1924, 1937, 1940), Ford (1922), Clark (1922, 1926), Hoffmann (1893, 1894, 1897, 1898, 1899), Holmgren (1922, 1939, 1940, 1941, 1942, 1943), El Toubi (1941, 1947, 1949, 1952), and Bonde (1945). But it will be seen that almost all the above studies were based on temperate water species, such as Squalus acantbias, Scyllium canicula, and Acanthias vulgaris, and various Skates and Rays. Though the study of these species have thrown much light on the embryology of Elasmobranchs of the temperate regions, so far no attempt has been made to find out whether those species which breed under tropical conditions
show any variation in their early developmental stages, from what has been observed in the case of temperate water species.

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. So the only references available are restricted to life-history studies and morphological descriptions of the embryos of a few sharks by Alcock (1890, 1892), Wood-Mason (1891), Wood-Mason and Alcock (1892), Southwell (1915), Southwell and Prashad (1919), Smedley (1926, 1927), Deraniyagala (1934, 1945), Aiyar and Malini (1936), Setna and Sarangdhar (1949, 1950) and Setna (1949).

The present work is therefore an attempt to give a detailed account of the early development of Chiloscyllium indicum, one of the egg-laying sharks of the Indian seas.

Material and method

The breeding season of Chiloscyllium indicum lasts from December to March. These sharks breed in the aquarium conditions only after being acclimatised for several years. In the aquarium at Trivandrum one female (45.5 cm) C. indicum measuring 10" in length started laying eggs.
Unfortunately that shark dies after laying about twenty eggs. A thorough study of its breeding behaviour could be made during the period. The female sharks carrying eggs are identified by the marks of bitings of the male sharks on the pectoral fins, by an enlarged abdomen, and by the swelling of the thyroid, which is visible externally as a rounded bulge below the lower jaw. Eggs are laid in pairs, in batches of four, one pair was followed by another pair, after few hours. The interval between the batches of eggs is ten days on an average. After the death of the shark, thirty mature female sharks and twenty mature male sharks were introduced into the aquarium tank, during the months July to August. But none of them laid eggs during the next breeding season. In the next year also none of them laid eggs. So various other means were tried to collect the eggs and one method was found most successful. During the egg laying season the gravid females of C. indicum identified by the features mentioned above, were collected from the fishing boats at the Central Institute of Fishery Technology, Cochin, and from the fishing boats which bring the catch to the fish curing yards at Vypeen Island at Ernakulam. C. indicum is very rare in the catches during the egg laying season, but if the females are caught and are of mature size, they are sure to contain two to four egg-cases in their uterus.
The egg-cases in the uterus were removed carefully, after opening the fish, and were then kept in a bottle of sea water. Though the sharks were dead by the time the collection was brought in from the fishing boats, the egg-cases were found to be in perfectly good condition. Unlike the egg-cases laid in the aquarium tank, the egg-cases removed from the sharks have a pale colour probably due to incomplete tanning, and these developed a deep brown colour after being exposed for few days.

After the days collection was over, the egg-cases were numbered with on small bits of plastic sheets stuck on to the adhesive filaments of the egg-cases. These marked egg-cases were then transferred to a perforated plastic container, and kept submerged in the harbour. Free access of sea-water was thus available to these eggs. All the eggs in the plastic container continued their development without interruption. The individual eggs of a pair taken from the same fish were reared under different conditions, one in the aquarium tank and the other in the plastic container, and the difference in their rate of growth was noted. After eight days the embryo of the egg in the tank measured 4.3 mm in length and the one in the plastic container measured 5.1 mm. Similarly on the ninth day the length of the embryos were 4.7 and 11.4 mm respectively.
The time taken for the eggs to hatch also differed in the two cases. Under aquarium conditions it took fifty-four to fifty-eight days to hatch, whereas in the plastic container it took only forty-six to forty-eight days to hatch out.

Another interesting observation made during the collection of eggs is that, four eggs collected from a dead and decayed shark, developed into young ones, when transferred to the plastic container hung at the harbour. This evidently shows that the egg-case protects the egg inside from external bacterial infection.

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. Heidenhein's iron haematoxylin was found to be the best nuclear stain for the early stages, and for the later stages Delafield's haematoxylin and eosin were used. Mallory's tripple stain was also used for the later stages.

Serial sections of the following stages were used for the present study:— blastoderm measuring 500μ in diameter; oval blastoderm stages of 1.4 mm and 2.0 mm in lengths along the long axis; embryonic rudiments
The oviducts also show the same general disposition as in other Elasmobranchs (PL. IV, Fig. 1). They consist of a pair of long muscular tubes extending the entire length of the abdominal cavity. Their anterior ends curve towards each other and meet in the median line behind the pericardium and open into the abdominal cavity by a single, median, elliptical, oviducal opening. The edges of the oviducal opening are thin and frilled (od. op). Each oviduct is demarcated into two regions, a short anterior and a long posterior part, the two being separated by a glandular swelling, the nidamental gland (nd. g). In a mature female measuring 58.0 cm in length, the anterior part of the oviduct is 33.0 mm long and 3.5 mm wide. The inner wall of this region is thrown into wavy longitudinal folds extending throughout its length.

The nidamental gland is more or less conical, with its tapering end directed backwards. The gland is approximately 40.0 mm long and the broadest anterior end is 25.0 mm across. Externally two distinct regions can be distinguished in it, in fresh specimens; an anterior short pinkish area, followed by a whitish region which forms the greater part of the gland. But when the gland is cut open (PL. IV, Fig. 3), three zones are discernible as in C. griseum (Malini, 1940) and in ovo-viviparous and viviparous Elasmobranchs (Prasad, 1944). The first zone
is the short pinkish areamarked internally by shallow transverse folds (al. z), followed by a lamellated middle zone (sh. z₁), in which the lamellae are arranged transversely. The third zone (sh. z₂) is the tapering part of the gland, which has a smooth inner surface, with shallow longitudinal folds towards the pointed end and two longitudinal grooves (gr), a dorsal shallow groove and a ventral deep groove. According to Nalini (1940), the first zone is the albumen secreting zone and the second and third, the anterior and posterior shell secreting zones respectively.

The nidamental gland is followed by the posterior part of the oviduct which is 35.0 mm long and 7.0 mm wide. Its inner surface is thrown into a series of longitudinal folds. The opening of the oviduct into the uterus is constricted by a powerful muscular sphinctre (Pl. IV, Fig. 1, mu. sp). The uterus (ut.) is an elongated tube 65.0 mm long and 15.0 mm in diameter when it is not distended with egg-cases. The wall of the uterus is elastic and its inner surface is traversed by a number of transverse folds.

In a mature female 50.0 cm long the ovary is 80.0 cm long and 25.0 mm wide and 35.0 mm thick along the dorso-ventral axis. It is suspended from the dorsal body wall by mesovarium. The number of ova in the ovary varies according to the size of the individual. In the specimen
described above (Pl. IV, Fig. 2) the ovary contained eight large, oval, bluish-green ova, each measuring on an average 20.0 mm along the long axis and 17.0 mm across, twelve smaller bluish-green ova of diverse sizes and a number of perfectly spherical very small pale yellow ova, the smallest measuring roughly 2.5 mm in diameter. Each ovum is enclosed in a follicle of the ovarian wall which juts into the lumen of the ovary. These follicles are attached to the inner surface of the ovarian wall ventrally by a narrow neck.

The follicular wall (Pl. IV, Fig. 4) consists of three distinct layers as described by Samuel (1945), namely, an inner follicular epithelium (granulosa) (fo.ep.), an intermediate layer called membrana propria (m.p.), and an outer theca which in turn can be distinguished into a compact inner region, the theca interna (Th. i), and an outer loosely organised theca externa (Th. e). In Myxine (Lyngnæs, 1936) and in one of the Teleosts, Rhodeus (Bretschnieder and Duyvene de sit, 1947), the follicular epithelium (granulosa) is formed of uniformly tall columnar cells, each with a spherical basal nucleus. These cells are provided with fine protoplasmic processes (p. pro) on their inner side. But in Spinax and Chimaera (Wallace, 1933) the cells of the follicular epithelium over the animal pole are flat, while those over the rest of the surface, are columnar, the height of the columnar cells being the
maximum at the vegetal pole. As in the latter example, in *C. indigum* also the follicular layer is thin and transparent in the region of the animal pole, while the rest of the epithelium is thick and opaque.

Closely applied to the outer surface of the follicular epithelium is the intermediate layer, the membrana propria, which is a very thin layer of elongated cells. The outer layer, namely, the theca is formed of fairly thick connective tissue richly supplied with blood. According to Samuel (1945) it is almost parenchymatic. The two regions of the theca, namely, the theca externa and the theca interna, are distinguishable in this species also, although there is no clear line of demarcation between the two. The inner region corresponding to the theca interna is fibrous and richly supplied with blood. The outer part of the theca is formed of a vacuolated protoplasmic layer which is nucleated, though cell boundaries are only faintly visible. Blood vessels from the ovarian wall enter the follicle along the neck and branch out in a fan-like manner between the membrana propria and the theca.

Unfertilized egg

A fully developed unfertilized egg when taken out of the follicle is enveloped by a thin nonstaining vitelline membrane (primary egg membrane) (PL. IV, Fig. 4, vt. m).
In *Scyllium*, Balfour (1878) describes two egg membranes, namely, an inner zona radiata and an outer homogeneous vitelline membrane. Giacomini (1896) also observed these two layers in Elasmobranch eggs. However in a mature egg according to Balfour (1885), both these membranes form a composite vitelline membrane. A similar composite vitelline membrane is seen in the unfertilized egg described here.

The vitelline membrane adheres closely to the outer surface of the thin ooplasmic membrane. At one end of the long axis of the egg, opposite the end where the neck of the follicle is attached to the ovarian wall, the peripheral ooplasm (pe.o) is thickened into a circular disc measuring approximately 2.5 mm in diameter. This protoplasmic disc which lies over the yolk appears whitish against the background of the bluish green yolk granules and is the precursor of the future blastoderm.

Close to the inner surface of the peripheral ooplasm there are a few irregularly disposed rows of small spherical granules. These are smaller than the yolk granules which constitute the bulk of the yolk mass. Moreover they do not stain as deeply as the latter. Since these lightly staining bodies appear close to the periphery of the egg mass, they may be termed the cortical granules (Y.gr). Balinsky (1963) describes similar cortical
granules in Acidians and Frogs, and he adds that these granules break up during fertilization and supply materials for the development of the fertilization membrane.

The yolk is formed of granules of varying size, some of them are oval and plate-like, while others are subangular. Scattered among the larger granules, there are also a few dot-like particles. The larger elements among the yolk mass may be termed the yolk platelets (y.pl.) and the smaller ones the yolk granules. All the granules and platelets, irrespective of their size and shape take up a deep stain with eosin.

When the ovum is fully developed the wall of the enveloping follicle ruptures and the egg is liberated into the body cavity with the vitelline membrane intact. From there it is moved towards the oviducal opening and enters the oviduct. Fertilization probably takes place in the anterior part of the oviduct, as in the case of C. griseum (Aiyar and Nalini, 1938).

According to Hobson (1930), once ovulation has taken place the passage of the egg down the anterior part of the oviduct and the formation of the shell is accomplished very quickly. After fertilization, as the egg passes through the oviduct the horny egg case is formed and thus when the egg reaches the uterus it is ready for
extrusion. In the uterus no further changes take place except that segmentation progresses rapidly during the passage through the uterus, and by the time the egg is extruded the blastoderm is fully formed. The stage of development reached by the egg depends on the time taken for extrusion. Usually the egg is extruded only when the parent finds a suitable place for attaching the egg, and so when the just extruded eggs are taken up and examined, they show different stages of development.