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by

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A definite study of the morphology of the sex glands of teleosts was begun nearly a century and a half ago by Cavolini (1792) who described not only the grosser structures but also some of the finer details. However, his work was so overshadowed by that of Rathke (1824) that it is now of but historic interest. Our first accurate knowledge of the form, structure, position, ducts, membranes and blood supply of the sex glands of the bony fish dates back to the classical work of Rathke. He distinguished between adult and immature testis, described the vas deferens, the structural arrangement of the ovary, and discovered the oviduct. Two years later Treviranus (1826) described for the first time the tubular nature of the teleostean testis. Vogt, in 1845, described spermatogenesis in the Salmon as well as the expulsion of ripe eggs. The next great contribution to our knowledge of the sex organs of fishes was made by Hyrtl in 1850. His work with respect to the ducts and accessory parts is especially valuable. Hyrtl and Rathke are the two outstanding early investigators in this field. Brock (1878) reviews the literature up to his time and makes many important contributions to the finer details and extended the studies over a wide range of species. Felix (1906) in Hertwig's Handbuch brings the literature down to more recent times, especially with respect to the development. Eigenmann (1896), Essenberg (1923), Böhi (1904), Turner (1919), Frances Clark (1925), and Hann (1927) have made the more important recent contributions to the subject and will be referred to later. Defosse (1856), Syrski (1876), Brock (1878), Stephan (1901), and Van Oardt (1929) are especially important in connection with the problem of hermaphroditism in bony fishes.

The common gold fish (*Carassius auratus*) has been chosen for this series of studies partly on account of its alleged

hermaphroditic tendencies, and partly because the material can be easily obtained and handled.

Most of the material for this investigation was obtained through the courtesy of the Bruce Gold Fish Fisheries at Thornburg, Iowa, but some fish were purchased from local dealers and some were obtained from garden pools. The specimens were kept in laboratory aquaria until needed. The eggs and young fry were kept in small battery jars aerated by a continuous stream of fine bubbles of air obtained by forcing the air from the supply pipes through short pieces of twig. The older fish were kept in a larger aquarium supplied with running water. No difficulty was experienced in keeping the fish in good condition in all of the stages. Most of the specimens were of the fan tail variety, although a number of shubunkins, telescope-eye, single fins, and grays (in which the larval gray had persisted up to the adult stage) were also used.

The youngest embryos were fixed entire, either in Bouin's or in Zenker's fluids. The older ones, from 10mm. up, were beheaded and the gonads exposed or removed entire, according to size, and fixed. Bouin's fluid proved a most satisfactory fixative for general use. Zenker's, Helly's, chrom-aceto-formaldehyde, 10 per cent and 20 per cent formaldehyde, Flemming's (without acetic), osmic acid, Cajal's and Champy's (formula II) fluids were also used and have advantages for certain features. Sections were cut from 4 to 10 micra in thickness, according to needs. The standard method of staining used was Heidenhain's iron haematoxylin (long method), counterstained lightly with congo red. The other methods of staining were as follows: Delafield's and Ehrlich's haematoxylin with various counterstains, Cajal's silver method, osmic acid, and a few other methods recommended for mitochondria and Golgi bodies.

All measurements are from the tip of the snout to the base of the tail.

The problem of the origin of the germ cells has been the inspiration of much investigation and is yet far from being completely solved. Several theories have been advanced concerning their origin. The earliest is that presented by Waldeyer (1870) and is still held by many eminent investigators. According to this view the germ cells are developed *in situ* by a transformation of the epithelial cells of the peritoneum in the region ventral to the mesonephroi. A second theory, the gonotome theory, in which the germ cells were thought to have been derived from segmental portions of the mesoderm was advanced by Rückert and others, but has had no great following. The theory of the early segregation of the germ cells seems to be the most popular at the present time. It has been advocated by Nussbaum ('80), Allen ('11), King ('08), Kuschakewitsch ('10), Witschi ('14), and many others.

In the fishes there appears to be a great deal of difference as to the exact time when the germ cells can first be definitely distinguished. According to Böhi ('04) the earliest germ cells are not found in the trout until 25 days after fertilization. At this time they are found in the splanchnopleure or in the somatopleure. Sixty days after fertilization they have arrived in the peritoneum, ventral to the mesonephroi, where they form a thickened band extending from the ninth to the thirty-second trunk segment. Three regions may be distinguished in this band; a middle region containing germ cells, a short anterior, progonal, region free of germ cells, and a postgonal region, also free of germ cells. Hann ('27) finds certain giant cells in Cottus embryos of less than 2mm. in length, a stage in which the optic vesicles and the notochord are just forming. These giant cells give rise to the germ cells, but also to ectoderm and mesoderm cells as well. Only those cells which come to occupy a favorable position become germ cells. Hann seems to favor the theory of the early segregation of germ cells, since, in Cottus, "the giant cells have never become differentiated into somatic cells they may be said to take their origin in indifferent cells." Nussbaum ('80) believed that he was able to distinguish germ cells at a very early stage of cleavage. Eigenmann ('91-96) traced the germ cells in Cymatogaster back to the fifth generation of cells.

In 4mm. gold fish embryos the germ cells are already easily distinguished in the dorsal peritoneum (Fig. 1). They stand out sharply defined from the peritoneal cells and cannot be

mistaken. The nuclei are large, elliptical, and lightly stained. They are not very abundant at this stage and are not uniformly distributed. Some are found at the root of the dorsal mesentery, or even in the mesentery itself. Others may be found in the dorsal celomic wall at a considerable distance away from the mid-line. Usually only one is found on each side in the same section, although in rare instances two or three may be found close together. At this time the germ cells do not project much into the celomic cavity, but seem to bulge as much above the thin peritoneal membrane as below it. Figure 1 shows a germ cell on each side of the mid-line with a third in the dorsal mesentery close to the pancreas. On some specimens the pigmentation of the peritoneal epithelium of this region is very heavy so that it is not always easy to be sure that some germ cells may not be hidden from view. The fact that germ cells are found scattered through the dorsal mesentery and at various points along the dorsal peritoneal wall would seem to favor the theory that the germ cells are migrating from the intestinal wall region by way of the dorsal mesentery.

In a slightly older stage, about 4.8mm. (Fig. 19), the germ cells are beginning to press down into the celomic cavity. The cell, itself, has not changed much in appearance, except that, perhaps, it is a little more rounded. The germ cells are now becoming aligned in preparation for the formation of the germinal ridge. Cranially the cells are disposed far laterad, but gradually converge mediad, caudally, marking out the course of the future gonads. A definite continuous band is not yet formed, but the individual cells project bead-like into the celomic cavity along its dorsal wall. Not all of the germ cells are collected in this band; some are still scattered along the dorsal wall toward the mid-line as though belated in their migration. The nuclei of the germ cells of this stage contain scattered chromatin granules. A distinct nucleolus is not evident. There are no indications of cell division and very little of growth. A germ cell of average size will measure about 148 microns in length and about 85 microns in width. The nucleus measures about 90 microns in the long diameter and about 37 microns in the short.

In embryos between 7 and 8 millimeters the germ cell has

pushed down into the celomic cavity so as to lie almost entirely below the peritoneum (Fig. 2). It has carried with it an investment of peritoneal cells and in the more advanced embryos, is attached to the celomic wall by a very short, thick mesogonium. The germ cell has become more spherical in shape, is much more definite and takes a deeper stain. A distinct nucleolus is now present and the chromatin material is scattered. Rounded, deeply staining bodies are arranged peripherally just inside the nuclear membrane and seem to be connected with the central nucleolus by delicate strands of linin fibres. The germinal ridge is becoming more definite although it dwindles to almost nothing between the germ cells, forming a sort of undulating ridge on each side of the mid-line, following the V-shaped path outlined in the earlier stage. Each germ cell is invested by a single layer of flattened peritoneal cells. On the dorsal side where it is attached to the celomic wall there may be a mass of several rounder and more deeply staining cells. These appear to be modified peritoneal cells and are the beginning of the stroma cells. These cells will be discussed more later. At this stage, also, the cytoplasm of the germ cells may contain one or more darkly staining basophile cells.

The germ cells now begin to multiply so that at the 10mm. stage (Fig. 3) two or three or more germ cells may be grouped together. They are large and about the same size. No mitotic figures are found. The number of stroma cells also increase rapidly, partly by multiplication and partly by invasion from without. The darkly staining, rounded nuclei of these cells distinguish them clearly from the other cells of this region. In the 11mm. larva (Figs. 4 and 25) as many as five or six germ cells may be found together in the same section. Connective tissue and stroma or cord cells occupy a large part of the gonad. Chromosomes are recognizable and are more or less scattered throughout the nucleus. Cell boundaries are becoming less distinct and the cytoplasm stains weakly. With the rapid increase of germ cells the gonad now begins to assume a club-shape in cross section and hangs freely in the celomic cavity where not pressed laterally by the digestive organs. In stages between 11mm. and 14 or 15mm. (Figs. 5, 6, and 21) the increase in size of the sex

gland is due more to the rapid multiplication of connective and interstitial cells than to the number of germ cells themselves. The germ cells become less crowded and are separated by the looser connective tissue so that the cell outlines show up more distinctly. Nucleolar granules are definitely lined up against the nuclear membrane and a distinct nucleolus is evident. The gonads are becoming more and more free from the celomic wall so that they are eventually connected to it by only a thin membrane, the mesogonium. The gonads are beginning to differentiate into ovaries and testis.

It is rather difficult to determine the exact time when sex-differentiation begins. Neither measurements nor days after fertilization are entirely satisfactory. Perhaps the time of spawning has an important effect. Those spawned in early May develop slower than those spawned in June due to climatic conditions. Specimens reared indoors in small aquaria and battery jars are smaller than those of the same age reared in the large out-of-door ponds. Other factors may enter in, such as the variety or breed, possibly the character of the food, and the oxygen supply. At least specimens of the same stage of gonadal development may vary as much as several millimeters in length and a number of days in age.

Sex differentiation manifests itself morphologically in several ways: (1) in the shape and general appearance of the gonad itself; (2) in the manner of the attachment of the gonad to the celomic wall; (3) in the character and arrangement of the efferent ducts; (4) in the character and arrangement of the rete cord cells; (5) in the size, arrangement, and appearance of the germ cells; (6) in the arrangement and appearance of the nuclear material; (7) in the presence or absence of a distinct nucleolus; and (8) in the blood supply.

In an embryo of about 15mm. (Figs. 7 and 8), the gonad has increased much in size and has become more vascular. A careful examination of a number of specimens of about this age indicates that sex-differentiation is beginning to take place. In some specimens as in figure 8, the germ cells are quite large and are more or less isolated, or tend to be arranged in rows. The nucleus is large, round, and clear. No distinct nucleolus is present, but the nucleolar material is collected into rounded, deeply staining bodies disposed peripherally against the nuclear membrane. The chromatin is scattered so as to be distinguishable, if at all, only at the nodes in the linin network. The cytoplasm appears as a rather narrow zone around the nucleus, not staining very deeply with acid stains. In some cells there is a very narrow, slightly darker staining zone of cytoplasm bordering the nucleus (Fig. 24). The gonad is somewhat elliptical in shape and is pressed closely against the celomic wall. The distal border is free of germ cells and the tissue is loose and vascular in nature. This is evidently the foreshadowing of the ovary. In other specimens of about the same stage of gonadal development (Figs. 5, 7, and 21), the germ cells are smaller and are more closely crowded into groups, or "nests." A distinct nucleolus is always present and rounded chromosomes are scattered throughout the nucleus. The gonad, as a whole, is more slender and club-shaped in cross section, and hangs freely in the celomic cavity except where crowded by the organs of the digestive tract. This type is evidently the anlage of the testis (Figs. 10 and 13).

In a female larva of a very early stage of differentiation, about 18 to 18.5mm. (Fig. 22) the gonad has become firmly attached to the celomic wall by its ventral border so as to form an enclosed cavity between it and the wall. This is the parovarial sac. Its enclosure begins near the anterior end and proceeds caudally along the entire glandular portion of the gonad. The postgerminal portion of the germinal ridge remains as a simple fold extending caudally, but gradually becoming smaller and smaller until it can no longer be distinguished from the peritoneum. It disappears completely a short distance craniad of the anal opening. The one on the left side disappears first. The progonal portion of the gonad extends forward a short distance as a low ridge but quickly melts into the peritoneum. At a little later stage the oviduct begins to be formed as the postgerminal or hypogonal fold broadens and becomes attached to the lateral celomic wall by its ventral border as in the formation of the parovarial sac, of which it is the direct continuation. This proceeds caudally, so that in a larva of 23.5mm., an enclosed

tube is found on each side which ends blindly a short distance in front of the anus.

The ovary of the 18-18.5mm. stage (Fig. 24) shows germ cells of various sizes so arranged that the smaller immature oocytes are located nearer the ovisacal border along with the oogonia, while the larger ones extend outward into the peripheral, more vascular region. The oogonia are undergoing extensive mitotic divisions so that in many places groups or nests of a dozen or more oogonia are massed together. In these the chromosomes are clumped together as in the bouquet stage of mitosis. The larger oocytes have large, rounded, lightly-staining nuclei without a distinct nucleolus and with the chromatin material distributed. Rounded nucleolar bodies are pressed against the nuclear membrane. Here and there these nucleolar bodies appear to be budding or dividing and sometimes a small bud is seen to be protruding through the nuclear membrane into the cytoplasm. Similar bodies of various sizes are found scattered through the cytoplasm on all sides of the nucleus. This would seem to indicate that nucleolar extrusions are taking place at this time and that they are moving through the cytoplasm toward the periphery of the cell where a little later they form a definite zone. Nucleolar extrusions have been described for a number of different forms. As early as in 1884, Will described the passage of nucleolar material into the cytoplasm in the eggs of Amphibians. This was further corroborated by Leidig (1888) and Macallum (1895). According to Narayan Rao (1928), his preparations of the ovarian ova of Loris: "Show nucleolar extrusions into the cytoplasm of the oocytes of different degrees of growth." "The appearance of the nucleolus in the nucleus, and the subsequent extrusion of parts of it into the general protoplasm, are associated with the phenomena of oogenesis and vitellogenesis." He further states that "In the tubal eggs the nucleolus is not indicated, and, indeed, its appearance is correlated with a certain size of the ovum and with its being surrounded by follicular cells, and the breaking up of and the redistribution of the chromatin threads of the nucleus itself."

Gardner (1927) finds that in the Horseshoe crab that the "nucleolus is found to arise by the confluence of substance

which passes from the cytosome into the nucleus" and suggests that the "chondriosomes, and possibly also the dictyosomes, are derived from an excess of this substance which accumulates in the cytosome." She also states that during oogenesis a greater part of the nucleolar material is passed back into the cytosome transporting with it phosphorus from the nucleus to the cytosome, where it is used in the synthesis of yolk. According to Gardner, "The definitive yolk arises by the interaction of nuclear emissions, chondriosomes, dictyosomes, and ground cytoplasm."

There are a number of problems connected with the origin, staining-reactions, variations in shape, and probable functions of these nucleolar bodies which will be considered later. Although the actual occurrence of nucleolar emissions into the cytoplasm has frequently been questioned, there seems to be no doubt that it does occur in the gold fish from a very early stage in sex-differentiation until vitellogenesis is completed. In sections that have not been properly fixed the nuclear contents may shrink away from the nuclear membrane carrying these bodies along. In this case it is not uncommon to find that the nucleolar bodies still retain connection with the extranuclear bud or button by a slender thread or tail, much as chewing gum will string out if a small bit is held by the teeth and the rest pulled away.

Another interesting feature presents itself in the 18-19mm. stage. The cytoplasm, which now begins to form a wider margin around the nucleus, also begins to show a zonation. In general,, the cytoplasm is somewhat coarsely granular in structure. There is, however, a narrow zone immediately surrounding the nucleus which is more finely granular and deeply staining than the peripheral portion. This perinuclear portion may be entirely absent in some cells of the 18mm. stage but in most cases it can be distinguished. Figure 24 shows the usual condition in the 18.5mm. stage. This is the beginning of a change in the chromophility of the cytoplasm and will be further discussed in connection with some of the later stages.

Figure 25 shows a section through the same gonad as figure 24, taken through the caudal region, just as the ovary is passing over into the oviduct. It will be noted that there are

still a few immature germ cells present, but that the wall is very much restricted in thickness and is denser. The large ovarian bloodvessel has become external and has acquired a rather firm wall.

The larva is now beginning to lose the slender typical fish form and is assuming the deeper gold-fish shape. It is also changing from the ancestral gray coloration to the reddish gold or silver white color, with intermediate gradations. A section through the upper region of the ovary of a 19mm. larva is shown in figure 26. One is struck at once with the rapid increase in the number of large oocytes which have appeared since the 18.5mm. stage. Most of the cell nests have disappeared, the cells having differentiated into oocytes of a larger size. There is still the arrangement of the largest oocytes in the peripheral more vascular zone, while the smaller oocytes and oogonia border the parovarial sac. An occasional large oocyte may be found undergoing mitotic division but it is not common. There may be, however, active division among the oogonia. The change in staining reaction of the cytoplasm is becoming more evident. The change from the acidophilic to the basophilic reaction was foreshadowed in the earlier stages by the zonation of the cytoplasm. This began around the periphery of the nucleus and gradually spread outward toward the margin of the cell. This zonation and change in chromotophily has been frequently described in literature and various explanations have been made to account for it. Robert Scharff (1888) found that the protoplasm of the egg of the haddock "shows a division into two distinct layers or zones, an outer lighter and an inner darker or denser one." The darker zone increases in width as the ova grow so that "in some instances the dark zone has invested the whole ovum, and the light portion has entirely disappeared." Even earlier than this Eimer (1872) and Bambeke (1875) had described a differentiation of the cytoplasm in the eggs of osseus fishes similar to this. Ludford (1921) describes chromophility in the cytoplasm of the oocyte of Patella. According to him, the transition from the undifferentiated cell to the oocyte is marked by a change in the chromophility of the cytoplasm from oxyphilia to basophilia. And that during the early stages of oogenesis, the cytoplasm remains basophil. A change

toward secondary oxyphilia occurs about the time that Golgi elements have become scattered and the mitochondria are commencing active division. From this stage onward, the cytoplasm becomes gradually more oxyphil until definite secondary oxyphilia is attained. According to Gatenby (1920), "In many cases it becomes possible to identify a thick halo of differentiated cytoplasm surrounding the nucleus. * * * One is irresistibly induced to believe that this halo is formed by materials squeezed, or at all events passively passing out of the nucleus." In the gold fish the beginning of this change in staining reaction starts with, or immediately following the beginning of nucleolar emissions and the returning to the acidophile condition takes place during vitellogenesis. A further discussion of this interesting phenomenon will be taken up in relation to vitellogenesis, in a later paper.

In gold fish larvae of about 23.5mm. the ovary has become much larger, due to the rapid increase in size of oocytes. It is somewhat elliptical in shape and is attached to the body wall by its ventral edge to form the parovarial sac. The germ cells seem to be arranged more definitely in columns radiating outward from ovisacal border toward peripheral or medial border. The smaller oocytes form a narrow zone adjacent to the parovarial sac, while the rest of the oocytes are approximately of the same size. The peripheral zone is comparatively free from oocytes, being made up mostly of connective tissue and bloodvessels. The arrangement of the germ cells adjacent to the central or lateral oviduct is common to most fishes. In the Cyclostomes, the gonad hangs down into the celomic cavity as a fold or plate, having germ cells distributed over the entire outer surface. The eggs are set free into the body cavity and pass out through the so-called abdominal pores to the exterior. In some of the Salmonoids, the germ cells become limited to the external, lateral surface and are set free into the body cavity. The medial surface, on the other hand, contains no germ cells, but is covered by peritoneal epithelium as though the mesovarium extended over to the ventral border of the ovary. In other teleosts, in which the ovary has a central cavity, entovarial sac, the germ cells border this cavity and the outer surface is clear of ova. In fishes having a parovarial sac, the arrangement of germ

cells is as in the gold fish. The arrangement is, therefore, essentially the same. The arrangement in the gold fish is the same as in the Salmonoids, except that the ventral border of the ovary is attached to the body wall instead of hanging free. In fish with an entovarial sac, the developmental history shows that the central cavity starts first as a groove on the lateral, germ cell bearing surface. This groove deepens and finally the edges come together and unite to form a tube. During the progress of development, the tube becomes central and is entirely surrounded by the oocyte-bearing zone. Thus, when the eggs are set free, they fall into this entovarial sac and out through the oviduct which is continuous with it. Figures 28, 29, and 30 and Plate 4 show sections through the ovary at various levels. Near the anterior end the ovary is large and filled with a large number of oocytes, becoming arranged in definite rows or plates in preparation for the formation of ovarian folds or lamellae. Caudally the ovary becomes smaller, the oocytes fewer and less regularly aligned. Finally the germ cells disappear and the ovary, one on each side, gradually passes over into the oviduct. The oviduct, like the ovary, has its lateral wall formed by the body wall, itself; while the medial wall is formed by the hypogonal portion of the germinal ridge. The oviducts converge toward the mid-line and unite near the caudal end of the mesonephroi, just as the mesonephric ducts are given off. The single oviduct runs for some distance between the ureters and the intestines as a duct of considerable size. It then suddenly becomes smaller and ends blindly without opening, either to the outside, or into the cloaca.

The question of the development and significance of the teleostean oviduct has been the subject of a great deal of study and of no little controversy ever since the time of Rathke in 1824. Rathke was one of the first to recognize the difference between the oviduct of the bony fishes and that of most other vertebrates. He found that in the eel and in some of the Salmonoids there is apparently no trace of an oviduct; but as in Petromyzon, the eggs are set free in the body cavity and pass out through the so-called "abdominal" pores. In some of the higher Salmonoids he finds that "there extends back beside each ovary a narrow band

which may be regarded as the remains of an oviduct. In all these fishes, therefore, the central abdominal cavity must take the place of the oviduct, as it receives the eggs when they are detached, and allows them to make their exit by a single opening at its posterior extremity." "In the smelts, however, there passes from each ovary a band, one edge of which is attached to the dorsal, the other to the abdominal wall, so that, in each lateral half of the abdominal cavity, there is a chamber which receives the eggs when they are detached from the ovary. The two chambers ultimately unite above the anus; and, in fact, close in front of the place where, in other fishes, the oviduct is situated." (Huxley.) The transition from this type to that of the gold fish is not difficult to imagine, if we consider that the amount of celomic cavity enclosed between the ovary and the ovarial fold becomes more and more constricted until finally a comparatively narrow oviduct and lateral ovisac is formed, with the body wall forming one half. The great difficulty lies in trying to harmonize the teleostean oviduct with the oviduct of most vertebrates. In vertebrates, aside from certain groups of fishes, the reproductive organs are more or less closely related to the excretory organs, both anatomically and genetically. Howe (81) classified the male genital ducts into two series: (a) the nephroorchidic, as in Elasmobranchii, Amphibia, and Amniota, where the vasa efferentia are present and the excretory organ is accessory to reproduction; and (b) the euthorchidic series, as in some Ganoids, Teleostei, Marsipibranchii, and Dipnoi, where vasa efferentia are not present and the Wolffian or segmental duct is exclusively renal in function. The female ducts may be classed in the same series. In series (a) the oviduct is a modified Müllerian duct; in series (b) the oviduct, when present, has an origin entirely independent of the true Müllerian duct. It would seem that in the Ganoids, Teleosts, and Dipnoi, the intrusion of the swim bladder between the region of the developing mesonephroi and the gonads has resulted in a complete separation of the urinary and genital systems. The testis in these forms does not discharge sperm by the way of the vasa efferentia through a modified mesonephric duct, and the oviduct is not a true Müllerian duct. According to Howe ('81) the Müllerian duct

invariably arises far forward, either in relation or immediate proximity to the head kidney, when such exists, and becomes completed, for the most part, by a slow backward extension. According to Jungersen ('93) the genital ducts of teleosts are peritoneal derivatives formed late, that is, subsequent to the differentiation of the genital gland, with the investment of which it becomes subsequently connected. One interpretation of this difference may be that the original Müllerian duct of the Elasmobranchs has degenerated in the line leading to the bony fishes, possibly in relation to the development of the swim bladder, and that the oviduct of these fishes is a new development. It is possible to reconstruct the possible history through the eels, the Salmonoids, and smelts to the entovarial and parovarial types common in the higher modern fishes.

In a young gold fish of 27mm. the ovary has become distinctly lobulated. Connective tissue and bloodvessels have invaded the spaces between the columns of germ cells forming longitudinal lamella which are united on the margin adjoining the ovisac, but are free distally. Oocytes of all stages are present and oogonia are scattered along the margins. The cytoplasm of the oocytes is entirely basophilic. No indication of cell division is present. The chromatin is completely scattered; no definite, central nucleolus can be seen, but the peripheral nucleolar bodies are very distinct and actively engaged in giving off portions into the cytoplasm.

In the 32mm. stage vitellogenesis is beginning to be evident. The yolk spherules are being formed peripherally; some ova show a complete peripheral ring of yolk spherules, around which are massed a great many mitochondria. The larger ova are beginning to stain lighter; that is, with the onset of yolk formation, the cytoplasm is becoming acidophilic again.

It is not the purpose of the present preliminary paper to go into the question of vitellogenesis and activities connected with it, but merely to sketch the general course of development of the gonads up to the mature stage.

In the 50mm. stage (Fig. 31) the lamelliform nature of the ovary is very clearly shown. The process of yolk formation has continued, adding yolk spherules from the periphery

inward toward the nucleus so that in a short time the entire cytoplasm becomes packed with yolk. The egg membranes are forming and the nuclear membrane is dissolved. The nucleolar bodies are no longer spherical, but assume irregular shapes as though breaking up, or in active ameboid movements.

The number of egg membranes present in the eggs of teleosts has been a matter of dispute. In the gold fish there is a very thin outer layer of cells surrounding the capsular or so-called vitelline membrane. The capsular membrane becomes deeply pigmented so as to form a broad black line around the ovum (Figs. 16 and 35). Inside of this there is an equally broad radially striated membrane, the zona radiata (Fig. 36), and inside of this the true vitelline membrane, demonstrated by Scharff ('87), Fulton ('98), and others.

The origin of these membranes is much in dispute. According to Balfour, in Scyllium, even before the follicular cells are formed, a delicate membrane, apparently "derived from the vitellus" appears which is in the nature of a vitelline membrane, and is the equivalent of the vitelline membrane of Leydig, the albuminous membrane of Gegenbauer, and the homogenous membrane of Schultz. Internal to it, there is a very faint differentiation of the outermost layer of the vitellus into a perforated or radially striated membrane of Schultz, the zona radiata. Later the zona radiata becomes thicker and much more distinct. Eigenmann ('90) classifies eggs into three groups: (1) eggs with a single membrane, the zona radiata: (a) of a uniform structure; (b) differentiated into an outer and inner layer; (2) eggs with a zona radiata and a thin, homogenous layer which may bear appendages; (3) eggs with a zona radiata produced by the vitellus, and a thick outer layer produced by secretion of the granulosa cells. In addition to these the "zonoid" or true inner vitelline membrane has also been described, underlying the zona radiata. According to Scharff, it is "semifluid, usually devoid of granules, and stains lightly. It disappears entirely in the ripe ovum." Some writers, however, regard the zonoid layer as an artifact. The gold fish belongs to the third group, having a thick outer or capsular membrane. It also seems to have a very definite

zonoid membrane as well.

The completion of yolk formation varies in different kinds of gold fish. The gray colored gold fish seem to mature somewhat earlier than the shubunkins or the fan tails. In some specimens measuring 47-50mm. some of the ova are almost completely filled with yolk (Fig. 39). In gold fish measuring 100-115mm. some of the ova seem to be mature. The egg membranes are fully formed, the nuclear membrane has disappeared, the nucleolar bodies are irregular in shape as though disintegrating or undergoing ameboid movement, and large rounded mitochondria are abundant among the yolk spherules. Some of the ova are undergoing degeneration. This is indicated by a breaking up of the dark outer pigmented capsular membrane, followed by a breaking up of the zona radiata and, apparently a diffusion of the materials throughout the egg substance. The mitochondria are spherical, large, and show up very distinctly among the yolk bodies which soon begin to disintegrate. Soon the egg is a mass of dark-staining granular substance that easily falls out of the sections during the process of preparing the slides (Figs. 37 and 38). There are many problems connected with the process of yolk formation and with egg degeneration which cannot be considered in this paper, which is intended only to give the general course of development up to the time of maturity.

The history of the development of the male gonads and germ cells will be but briefly sketched at this time. Sex-differentiation begins in the gold fish between the 14 and 18mm. stage. Usually at 16 or 17mm. differentiation will have gone far enough so that it is possible to identify the sex. Figure 41 is a photomicrograph through a gonad of a 19mm. goldfish. It was hatched on June 15 and killed and fixed October 13. The gonad by this time has attained considerable size and is somewhat club-shaped in cross section. It is distinguished from the ovary of about the same stage of development by the broader attachment to the dorsal celomic wall, by the smaller, much more abundant germ cells, and the greater compactness. There is a less amount of interstitial tissue and the bloodvessels are smaller. The connective tissue septa wind about the germ cells segregating them into cell nests or lobules. Figure 9 shows the general arrangement

in a somewhat older stage. Each cell has a distinct central nucleolus and round chromatin bodies are scattered throughout the nucleus as shown in figures 11-13. Many of the germ cells are undergoing active mitosis which may account for the formation of the cell nests. In older stages, about 25-27mm. (Figs. 42 and 45), the spermatocytes are becoming arranged around a central lumen to form the seminiferous tubules. This rearrangement of cells together with the increase in numbers causes the testis to become more elliptical or egg shaped. There is still considerable evidence of mitosis going on in the various regions of the testis. In figure 44, showing a section through the testis of a 41mm. gold fish, the tubules have been definitely formed and a few of them are beginning to collect spermia. In the 50mm. stage most of the tubules have become filled with spermia. Figure 14 is from a gold fish spawned in early June and fixed December 7th. The seminiferous tubules are filled with spermia. A small ovum is found near the center of the figure. Figure 40 is a photomicrograph of a portion of the testis of a somewhat younger gold fish, measuring about 34mm. showing a small ovum isolated among the seminiferous tubules. Many of these isolated ova soon undergo degeneration and disappear. The question of hermaphroditism and juvenile hermaphroditism in the gold fish will be taken up at a later time. Figure 15 is a drawing of a few seminiferous tubules from the testis of a 26mm. gold fish to show the general appearance of the germ cells, the tubules, and the spermia of a stage when the spermia are just beginning to form. Figure 43 shows the conditions in the spring of the year. The fish at this stage measures from 112-115mm. in length and was killed and fixed in the early part of April. The spermia have so completely distended the tubules that their walls have become very thin and the interstitial tissue reduced to the minimum. At this time, not only are the intratesticular tubules filled with spermia, but the collecting tubules in the mesorchium and in the dorsal celomic walls are also completely filled.

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EXPLANATION OF PLATES

Plate I

Figure 1. Cross section of peritoneum and dorsal mesentery of gold fish embryo of 4mm., showing three germ cells, a small portion of the pancreas and a portion of the intestinal wall.

Figure 2. Gonad of gold fish embryo No. V, about 8.8mm., showing germ cell surrounded by a capsule of peritoneal cells projecting into celomic cavity.

Figure 3. Gonad of gold fish embryo No. 2, about 10mm., showing two germ cells, stroma cells, and capsule.

Figure 4. Gonad of gold fish embryo of about 11mm., showing several germ cells, capsular cells, stroma cells, and nuclear structure.

Figure 5. Gonad of gold fish embryo No. 59, about 12mm., showing increase in size due to connective tissue and stroma cells increasing.

Figure 6. Gonad of gold fish No. 46, about 15mm.

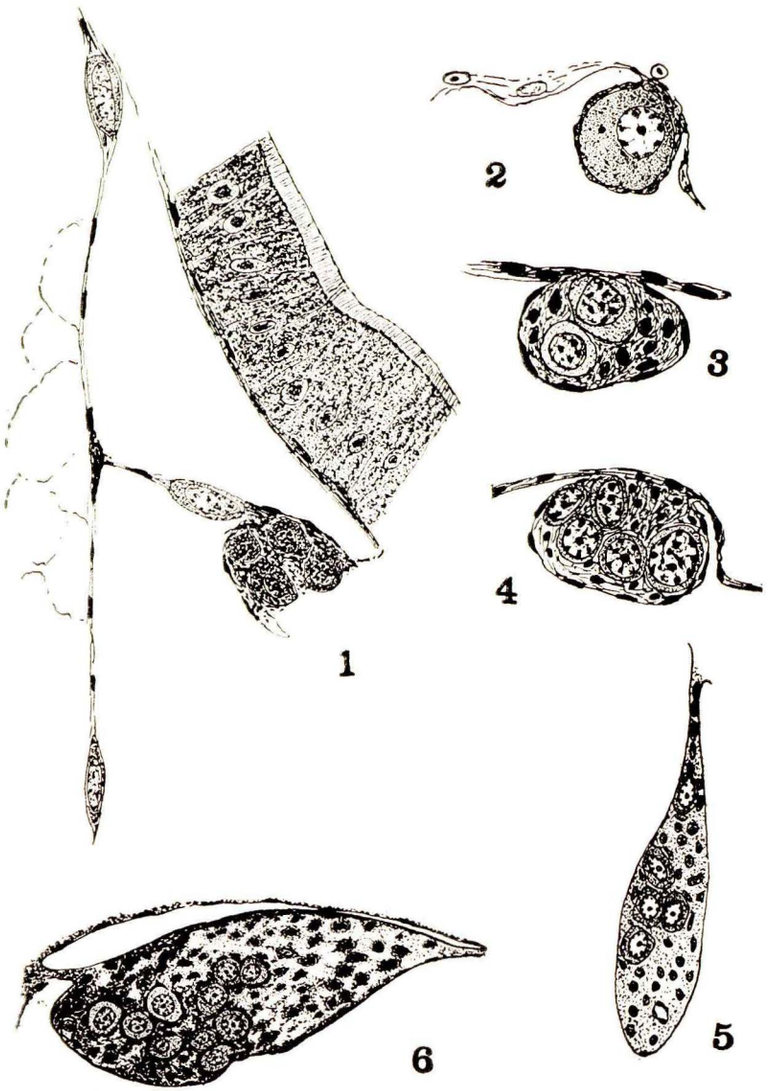


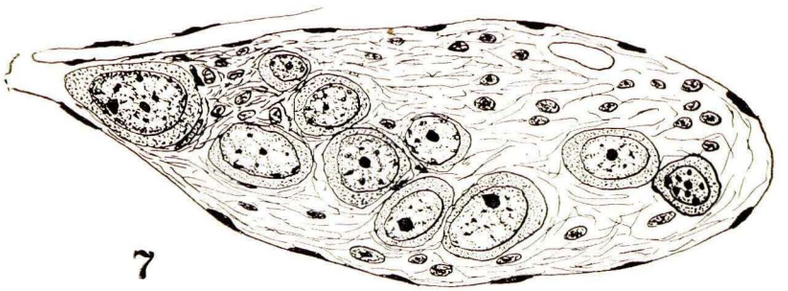
Plate I

Plate II

Figure 7. A gonad of a 12mm. gold fish, under higher magnification to show more detail. Series No. 19.

Figure 8. Gonad of 17.5mm. gold fish, series No. 64.

Figure 9. Section through a young testis to show nests of cells, character of nuclei, showing distinct nucleoli and scattered chromosomes.



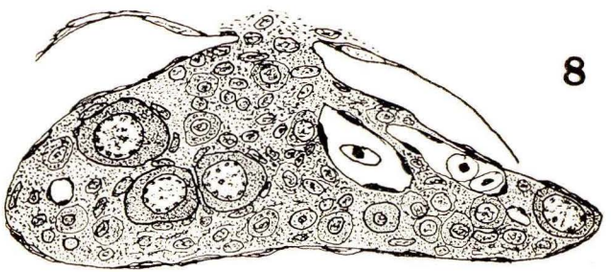


Plate II

Plate III

Figure 10. Section through the testis of a 21mm. gold fish, showing broad attachment to dorsal wall, the compact arrangement of cells, and the tendency to form cell nests or lobules.

Figures 11, 12, and 13 show small areas drawn under oil immersion to show details of nucleus.

Figure 14. Section through a testis from a gold fish of 26.5mm. length, showing tubules filled with maturing spermia. This specimen had been reared from time of hatching in a small aquarium indoors, so that it is really older than the length indicates. The fish was killed and fixed December 7. Note egg cell near center of testis.

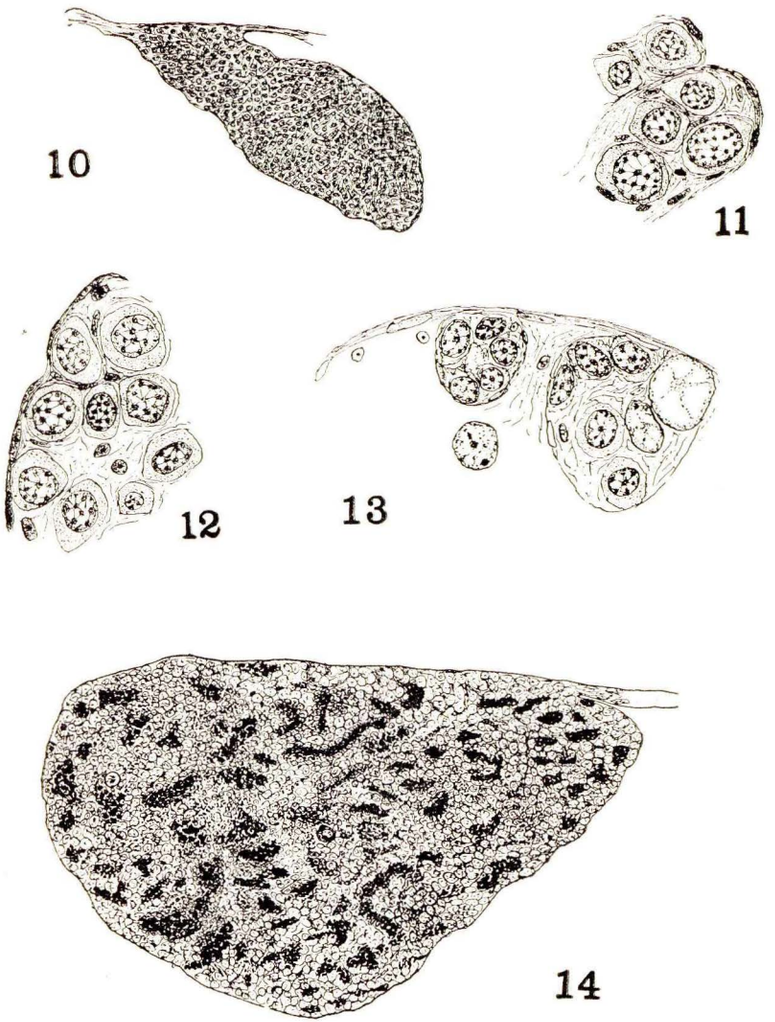


Plate III

Plate IV

A series of drawings taken through several levels of an ovary from a 23.5mm. gold fish. Section "a" from near the anterior end, and section "d" near the caudal end. Section "e" shows the two oviducts about to unite and the mesonephric ducts (M. D.) just leaving the mesonephros. Section "f" is taken a short distance anterior to the point where the oviducts (O.D.) end blindly. The mesonephric ducts have also united to form a single duct (M.D.).

Plate IV

Plate V

Figure 15. Shows a few tubules filled with sperm from a testis of a 26mm. gold fish. Series 16.

Figure 16. Maturing ovum from a 115mm. gold fish to show egg membranes, yolk spheres, and nucleolar bodies.

Figures 17 and 18. Oocytes from a 27mm. gold fish, series 7 shown in outline above. Shows nucleolar bodies and general appearance of cell.

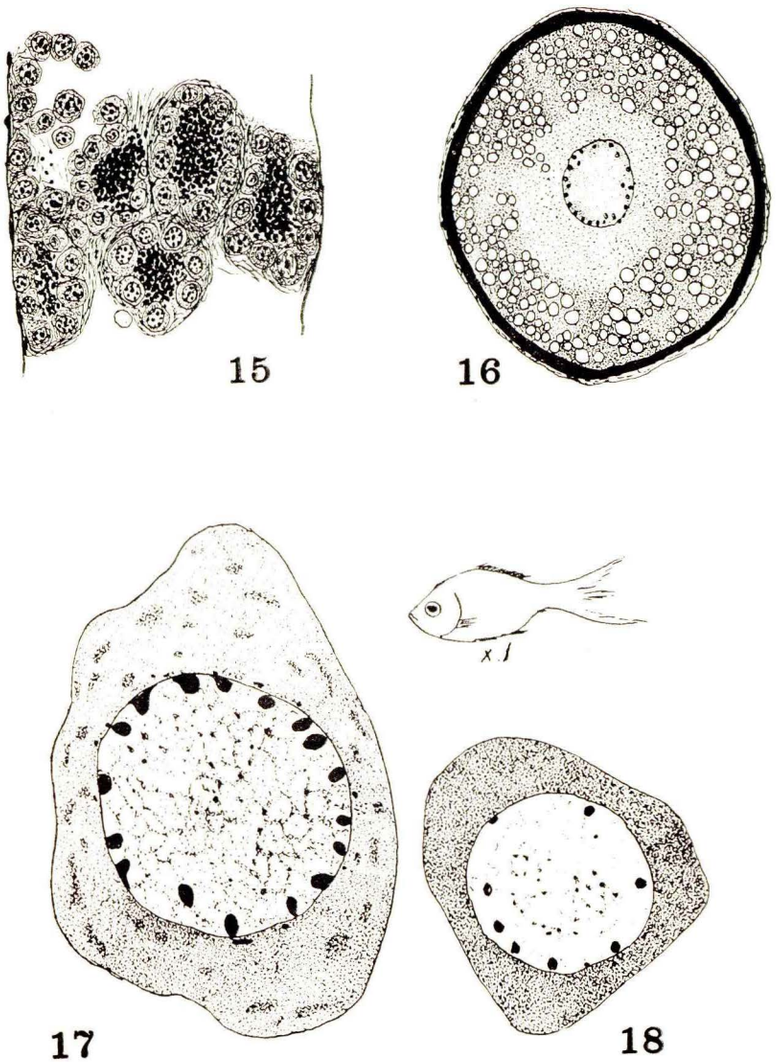


Plate V

Plate VI

Photomicrographs showing development of gonads

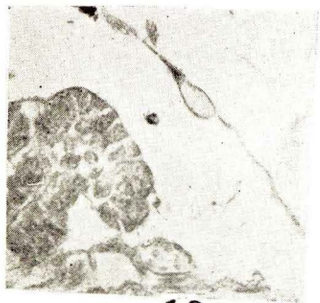
Figure 19. A section through the gonadal region of a 4.8mm. gold fish embryo, showing the germ cell just beginning to push into the celomic cavity; a slightly older stage than shown in figure 1.

Figure 20. Photomicrograph of the gonad of a gold fish embryo of 11mm.

Figure 21. Photomicrograph of a stage slightly older than the one shown in figure 6. Note one cell undergoing mitosis.

Figure 22. Photomicrograph through a young ovary from an 18mm. gold fish. Parovarial sac has formed.

Figure 23. Photomicrograph of a young ovary showing an oocyte with two nuclei.



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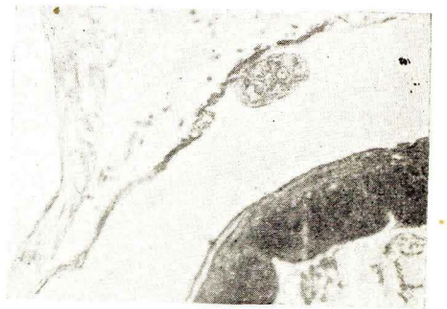

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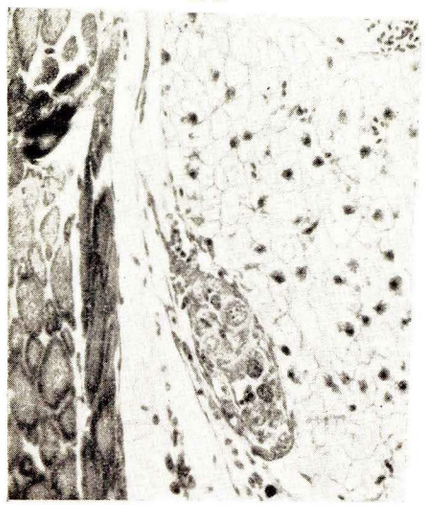

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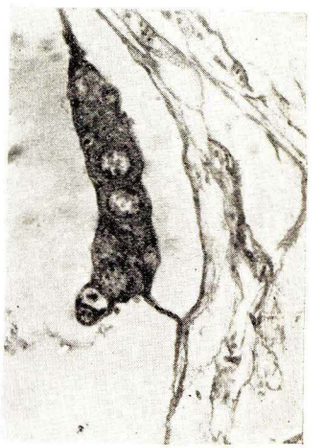

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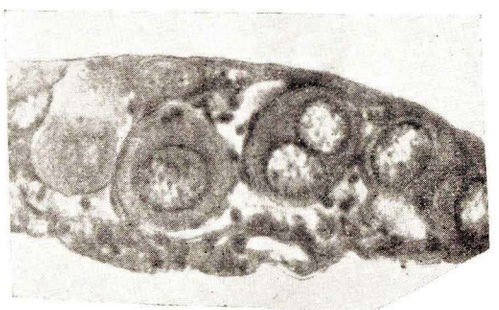

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Plate VI

Plate VII

Figure 24. Photomicrograph of a section taken through the upper part of an ovary from a gold fish of about 19mm.

Figure 25. Photomicrograph through the same ovary just before the ovary passes into the oviduct.

Figure 26. Photomicrograph through the anterior region of a slightly older ovary.

Figure 27. Photomicrograph through the ovary of a 50mm. gold fish, showing the ovarian lamellae and ova in various stages of development. Note difference of chromatophily in oocytes of different stages.

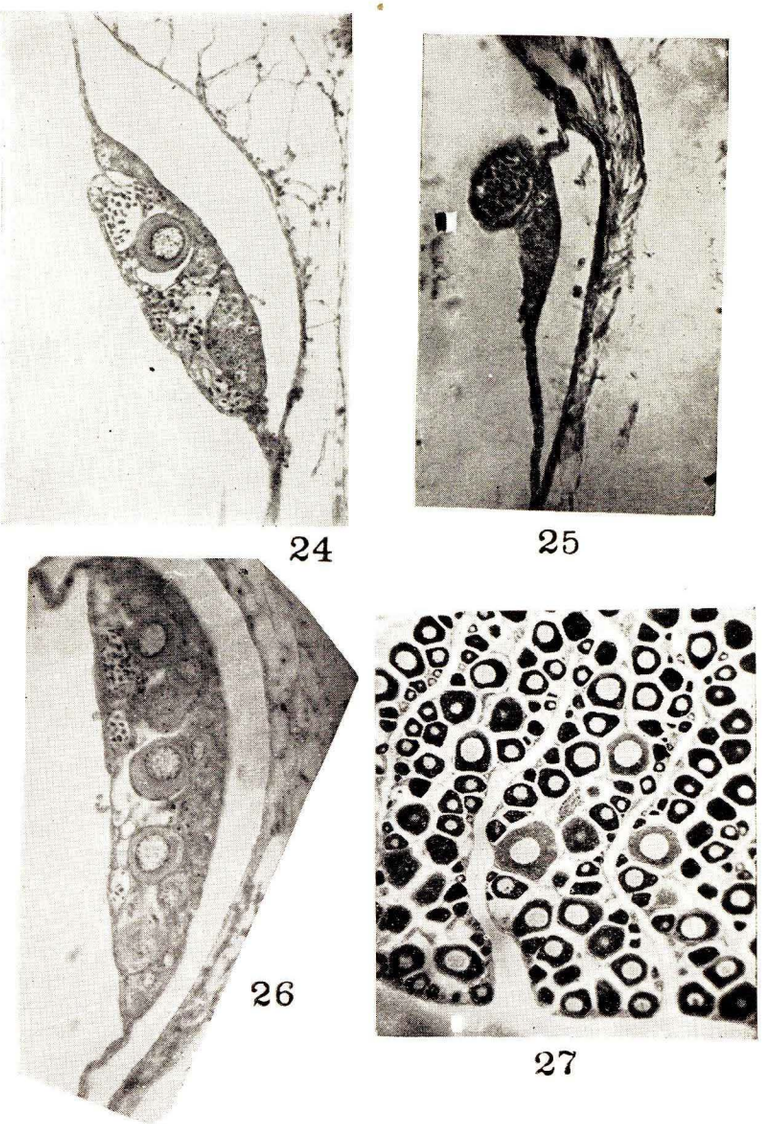


Plate VII

Plate VIII

Figure 28. Photomicrograph of a section through the anterior portion of an ovary from a 23.5mm. gold fish.

Figure 29. Photomicrograph through the middle region of the same ovary.

Figure 30. Photograph through the oviduct of the same gold fish. M., mesonephric duct; O., oviduct; I., intestine.
Photomicrograph through the ovary of a 115mm. gold fish, showing ova in various stages of development and yolk formation.

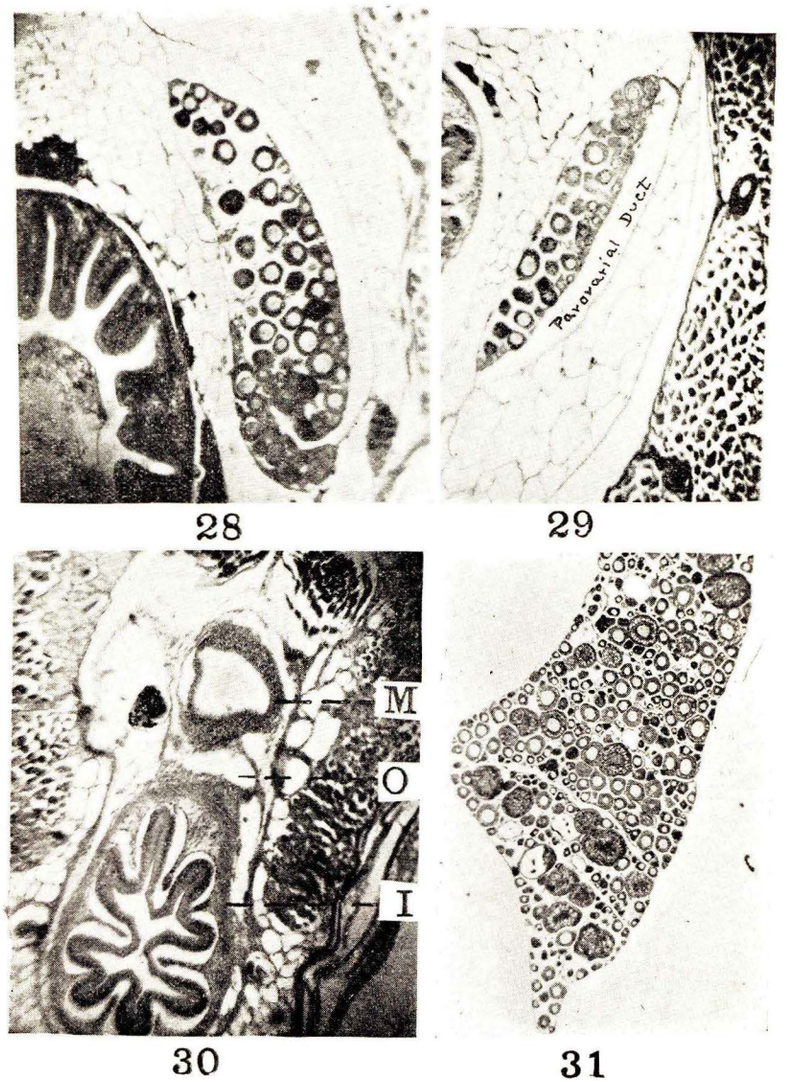


Plate VIII

Plate IX

Photomicrographs showing various stages in oogenesis

Figure 32. Oocytes showing active nucleolar emissions into the cytoplasm.

Figure 33. Several younger oocytes showing arrangement of nucleolar bodies.

Figure 34. Several oocytes showing chromatophily of cytoplasm and the beginning of vitellogenesis.

Figure 35. Section through an ovary showing one cell approaching maturity. Note the deeply pigmented capsular egg membrane. Above this is an egg undergoing degeneration and at the side, the remains of a "corpus luteum."

Figure 36. Portion of a mature ovum just beginning degeneration. Striations may be seen in the zona radiata. The pigment has gone from the capsular layer. Great numbers of large mitochondria are found around the disintegrating yolk spheres.

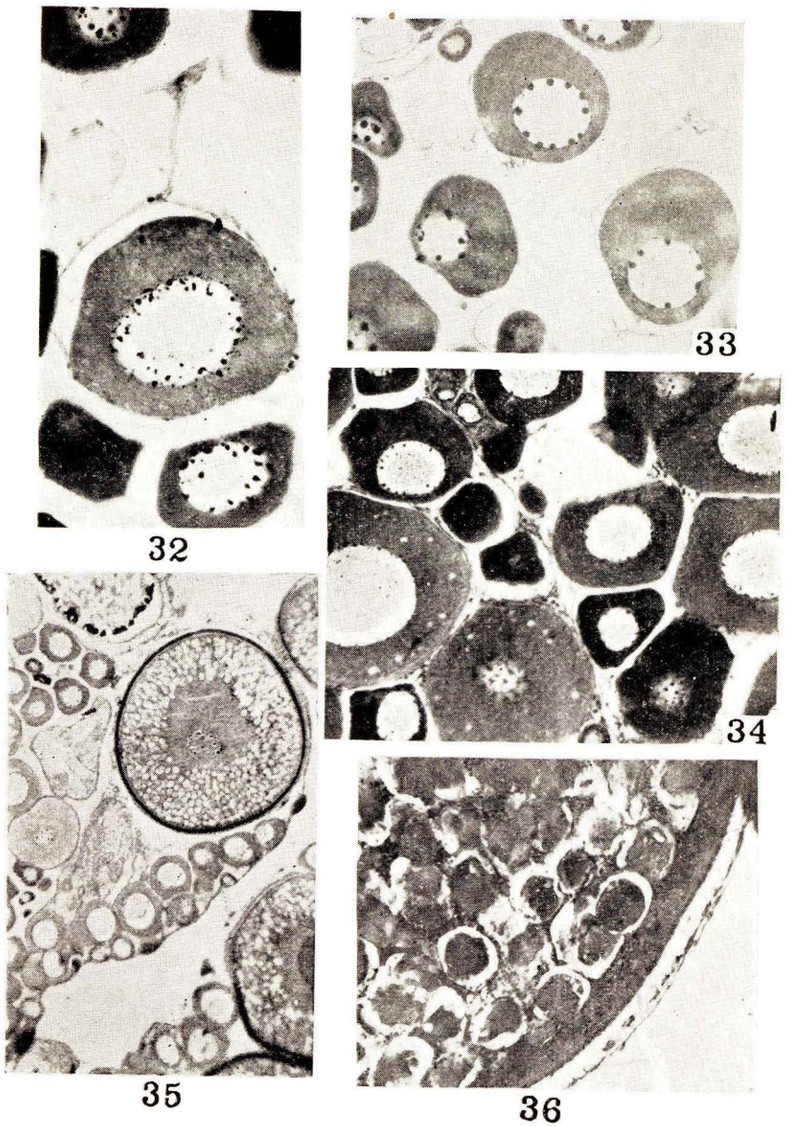


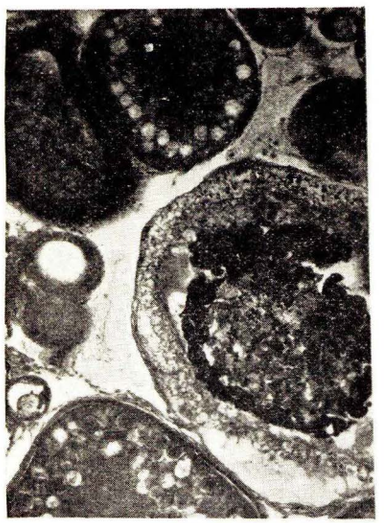
Plate IX

Plate X

Figures 37 and 38. Photomicrographs of ova in various stages of degeneration.

Figure 39. Section through the lower end of a mature ovary showing all stages of oogenesis and one or two degenerating ova.

Figure 40. Section through a young testis to show a solitary ovum among the spermatic tubules.



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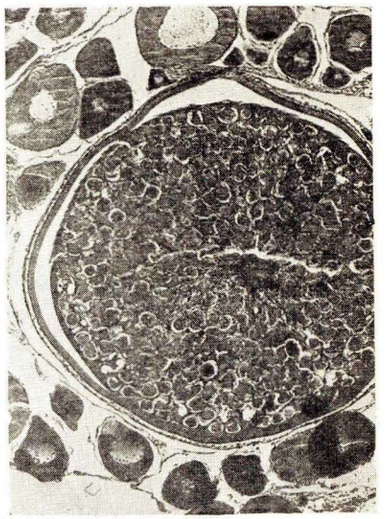

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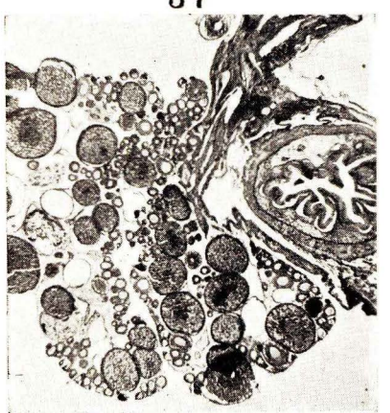

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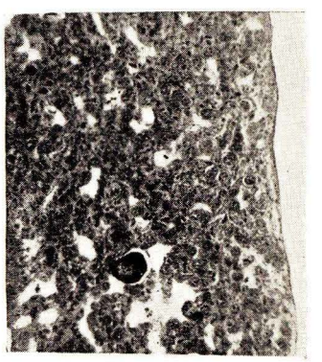

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Plate X

Plate XI

Photomicrographs to show stages in development of testis

Figure 41. Section through the gonad of a gold fish embryo of about 19mm. Note the broad connection with dorsal wall and the compact arrangement of cells.

Figure 42. Section through the testis of a 25mm. gold fish.

Figure 43. Section through the gonad of a 26mm. gold fish taken through the middle region.

Figure 44. Section through the testis of a 41mm. gold fish showing the tubules and sperm ducts in the mesorchium and dorsal wall.

Figure 45. Section taken through the region of the mesorchium of a testis of the same stage as figure 42 under high power to show details.

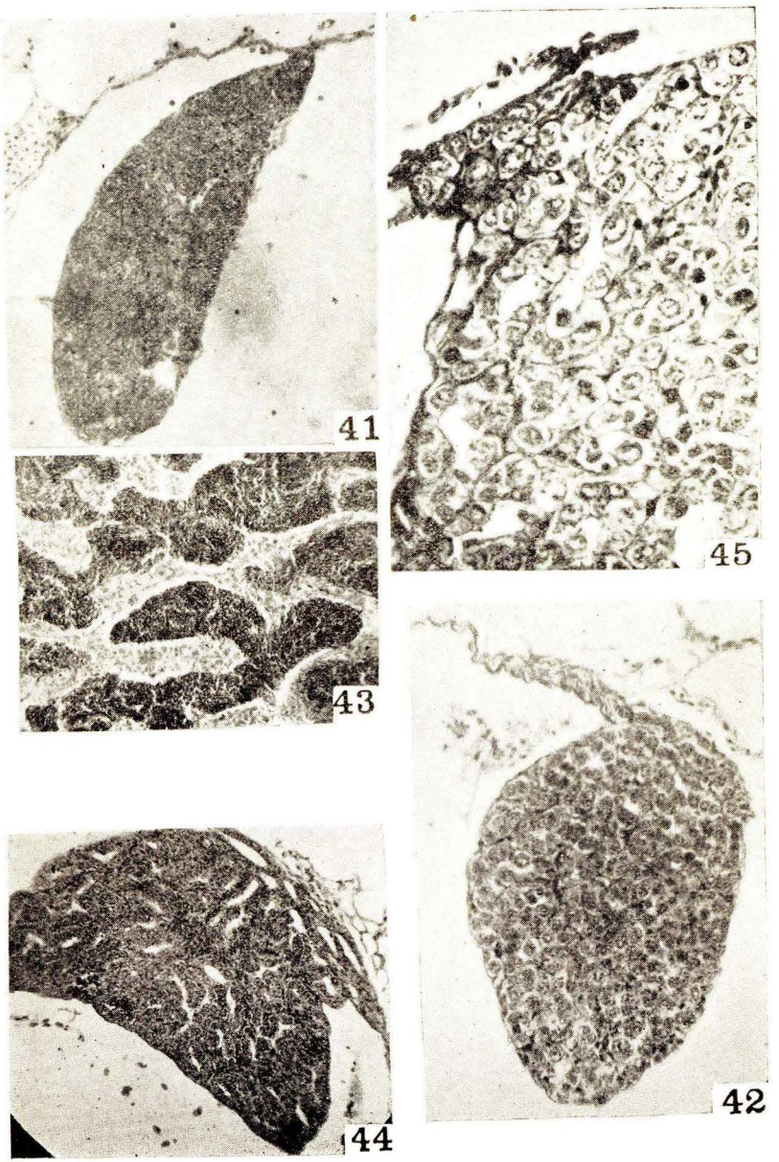

Plate XI