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EARLY DEVELOPMENT OF THE LARGEMOUTH BLACK BASS, MICROPTERUS SALMOIDES SALMOIDES (LACEPEDE), AND THE HISTORY OF THE GERM CELLS THROUGH THE PERIOD OF SEX DIFFERENTIATION

by
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INTRODUCTION

A mass of literature has accumulated dealing with the early history of the germ cells in animals and while the initial studies were concerned with invertebrate groups, some of the more complete ones have dealt with vertebrates. Interest in this problem was stimulated when it seemed imperative that the germ cells must remain unchanged from generation to generation in order to pass on the hereditary characteristics of the species. Theoretically, therefore, the germ cells must be set aside at some early stage of development and withheld from the assumption of any somatic duties.

While it is possible to trace the germ cells back to extremely early stages of development in certain of the invertebrates (Ascaris, Miastor, Sagitta and others) such is not the case for the vertebrates. The only example of such an early segregation for these forms is that of Micrometrus aggregatus reported by Eigenmann in 1891. In this form, it was claimed by Eigenmann that the germ cells could be recognized as distinct from the soma as early as the fifth or sixth segmentation. Although others have tried, none has succeeded in the years since to trace the germ cells so far back in the ontogeny of a vertebrate species.

Whereas numerous accounts dealing with the germ cell history in fishes have appeared in literature, none that approaches completeness has dealt with a representative of the sunfish family, the Centrarchidae. Recognizing the need of a careful and complete morphological study of the germ cells in a member of this group, this investigation was undertaken. The embryonic stages of the largemouth black bass, Micropterus salmoides salmoides (Lacepede) were used to determine the stage of development during which the primordial germ cells could be first

recognized and then to record their subsequent history up to the period of sex differentiation.

Although the present investigation was built around the history of the germ cells it was soon discovered that an understanding of their early development was dependent on a thorough knowledge of the early embryonic stages of the animal. The first part of the investigation is, then, concerned with the early developmental history of the bass. In spite of the economic importance of the bony fishes, very little work has been done on their early development. One must rely primarily on the classic work of H. V. Wilson on the sea bass, Serranus atrarius, which appeared in 1891, and to a lesser degree on those of Lereboullet (1854), Kupffer (1868), Oellacher (1883), Hoffmann (1884), Henneguy (1888), Schwartz (1889), and the more recent works of Oppenheimer (1937), Solberg (1939), Battle (1940, '44) and Carr (1942). Little help can be found in most standard texts on embryology but some may be obtained from the texts of Balfour (1881) and Ziegler (1902).

The work is presented in two parts: Part I Embryogeny and Part II The History of the Germ Cells.

MATERIALS AND METHODS

Most of the material used in this investigation came from the U. S. Fish and Wildlife Service Hatchery at Northville, Michigan. Additional collections of unaged fry and fingerling were made from the Huron River and from Whitmore Lake, both in Washtenaw County, Michigan.

During the period of 1946-48, one of the hatchery ponds at Northville Hatchery was made available for experimental purposes. Different groups of breeding adults were placed in the pond each year.

Four series of developmental stages were collected for study; the first in the spring of 1946, the second and third in the spring of 1947, and the fourth in the spring of 1948. Freshly spawned eggs were siphoned from the nests by means of a glass tube. A pair of long-handled lawn trimmers was employed to snip off rootlets with adhering eggs. The first series was collected and fixed at the nesting sites, while the second, third and fourth series were reared and hatched in the laboratory. The first series was composed of stages ranging from the freshly laid egg to young of two to three inches in length, the second and third series were made up of embryos fixed every two hours until hatching, and the fourth series consisted of embryos fixed at two hour intervals up to the eighteenth hour and then every four hours until hatching.

Several fixatives were employed. Among these were Benda's fluid, Bouin's, Allen's modification of Bouin's with chromic acid, Meves' solution, 10 per cent formalin and various strengths of alcohol. While none of these gave perfect results as yolk fixatives, the best cellular fixation was obtained with Bouin's to which had been added one gram of urea crystals per 105 parts.

Three methods of dehydration and clearing were used: the conventional

xylol and alcohol method, N-Butyl alcohol, and Dioxane. The best results were obtained with Dioxane. Because Dioxane is miscible in all proportions with water, alcohol, and xylol and is a paraffin solvent, dehydration and clearing can be combined in one operation. It was found that whole embryos could be transferred directly from the fixative into Dioxane. After a period of time, varying in length with the size of the embryos, direct transfer could be made into paraffin. While in paraffin, care was taken to prevent them from resting on the bottom of the container. Bituminous assay crucibles made of glazed porcelain were used for this purpose. This was necessary because Dioxane, although a paraffin solvent, has a greater specific gravity than melted paraffin and sinks to the bottom of the dish. Thus, if the embryos are not supported above the bottom, little or no infiltration of paraffin can take place. Best results were obtained when two or three changes of paraffin were made. One of the many advantages of the Dioxane method is that tissues may be left for hours or even days in the Dioxane-paraffin mixtures without becoming excessively brittle.

After infiltration, the embryos were embedded in Fisher Tissuemat, with a melting point of 56-58 degrees C., and sectioned at 8 and 10 micra, the majority being cut at 10 micra. More than two thousand preparations were made.

Complete serial sections of all prehatching stages were mounted and stained. This procedure was extended to include posthatching stages up to 13 millimeters in length. Subsequently, only the trunk portion was used. In larger specimens, fingerlings and up, only the swimbladder and the attached gonads were prepared for study.

The four stains used were: Harris' Hematoxylin, Heidenhain's Iron

Hematoxylin, Mallory's triple, and Heidenhain's Azan. Harris' Hematoxylin and Heidenhain's Iron Hematoxylin were most frequently used and although it has been claimed that Dioxane impairs the staining quality of the nucleus when stained with Harris' Hematoxylin, I encountered no such difficulty.

To designate the developmental stage for prehatching embryos the age in hours was employed, and for posthatching larvae, fry and fingerlings the total length in millimeters or centimeters.

NATURAL HISTORY NOTES

The largemouth black bass, Micropterus salmoides salmoides, is one of the most popular fresh water game fishes. Its spawning and nesting habits have been observed and reported by Lamkin (1901) in Georgia, Lydell (1904) and Reighard (1906) in Michigan, Breder (1936), and more recently by Carr (1942) in Florida.

I observed nest construction to follow the general pattern employed by centrarchid fishes. The males prepare the nest and guard the eggs during incubation. Most of the nests observed at the hatchery were constructed on gravel plots which had been placed in the ponds when dry. The water depth above them usually ranged from two and one half to four feet. Nest construction was observed on numerous occasions. At some sites where the gravel plots were overgrown with Chara the eggs were deposited on its stems and rootlets. Several nests were constructed near the bank in water eight to twelve inches deep. Such nests usually were made against the south bank beneath overhanging cattails (Typha). As a rule these nests were constructed by the younger brood stock while the older fish utilized the gravel plots. The nests near the bank could be observed with a minimum of disturbance to the male guarding them and at times it was possible to remove eggs without disturbing him perceptibly.

Spawning occurred when the water temperature approached 62 degrees F and ordinarily took place during the early morning or late afternoon. On one cloudy warm day one pair was observed to be spawning at about noon. The spawning activities of this pair were unusual and warrant description. The pair approached a bank nest beneath the cattails and the very vigorous male made body passes at the female, nudging her gently

but firmly with his snout from the head backwards. After several such passes he settled beside her and slowly spawning took place. When spawning was completed the female left the nesting site and the male began his parental duty of caring for the eggs.

In the nest described above, hatching took place in about fifty-six hours. Eggs spawned in the evening hatched more slowly, requiring from seventy to seventy-seven hours. Carr (1942) described a variety of nesting sites used by the bass in the wild and reported that hatching took from two to three days.

In the present study it was found that spawning was at its height between the fifteenth and twenty-second of May in each year that the pond was under observation.

PART I EMBRYOGENY OF MICROPTERUS SALMOIDES SALMOIDES

Although Carr (1942) has published an account of the embryology of the largemouth bass, certain phases of its development are presented here as a basis for a better understanding of the embryonic history of the germ cells. Among the phases to be considered are: the egg and the formation of the blastoderm; the origin and possible function of the periblast; gastrulation; germ layer formation, the origin of Kupffer's vesicle; the formation of the gut, coelom, and kidney ducts; and the relationships of the subintestinal yolksac extension.

For an account of larval development and certain other features, Carr's paper should be consulted. The development of Micropterus follows in general that of Serranus as described by Wilson (1891).

The Egg

The egg of the largemouth black bass is spherical, small, adhesive when first deposited and demersal. It varies in diameter from 1.5 to 1.65 millimeters and is surrounded by a tough flexible membrane, the so-called chorion, which is approximately .025 millimeters in thickness (fig. 10, ch.). Within this membrane, and apparently an integral part of it, may be found a zona radiata (Reighard 1906) or vitelline membrane (Solberg 1939). In addition to the zona radiata, two other membranes or layers are discernible within the chorion. One is homogeneous and lies on the inside and the other is granular and lies between the homogeneous layer and the zona radiata. The perivitelline space enclosed by this composite membrane is filled with a colorless fluid which coagulates when mixed with water. The bulk of the egg proper consists of an opaque

sphere of granular yolk surrounded by a delicate plasma membrane. After fertilization the yolk seemingly becomes more transparent. Embedded within the yolk and at one side is a large amber colored oil globule approximately 0.70 millimeters in diameter. The egg is free to rotate within the chorion and when deposited becomes oriented with the oil globule uppermost.

Segmentation and Blastulation

A few minutes after fertilization there is a streaming movement of the egg protoplasm toward one side of the yolk sphere where it collects and forms a lenticular cap, the polar cap (Solberg 1939) or blastodisc (fig. 1, bd.). In approximately half an hour the blastodisc swells into a bulging cap which thins out peripherally and the yolk sphere flattens beneath it. The oil globule, now at one side of the disc, marks the position of the future dorsal lip of the blastopore.

Within an hour after fertilization the first cleavage furrow appears. This furrow, a vertical one, originates in the center of the disc and extends rapidly towards the periphery. The cleavage, however, does not continue into the yolk and the blastodisc is thus divided into two almost equal blastomeres still united on their lower surfaces (fig. 2). Henceforth, the cleaving blastodisc may be considered as the blastoderm. The second furrow appears a short time later and is at right angles to the first. The eight-cell stage is formed by two furrows crossing the blastomeres parallel to the first, and the sixteen-cell stage is produced by two furrows parallel to the second. Horizontal and vertical cleavages follow in such rapid succession that it is difficult to trace them beyond the eight- and sixteen-cell stages. The early blastoderm is

oriented with its long axis at right angles to the oil globule and its blastomeres are approximately equal in size but become more irregular as cleavage continues. During cleavage no segmentation cavity appears as is found in amphioxus, some fishes, and other vertebrate types. However, we may assume the presence of a virtual blastocoel between the blastoderm and the yolk. Thus, a stage comparable to a blastula is attained when the blastoderm is several cell layers in thickness and is composed of outwardly similar cells. Wilson (1891) described the formation of a segmentation cavity for Serranus. According to him, this cavity was formed when the blastomeres remained in close connection at their upper surfaces but were loosely joined at their lower surfaces so that a cavity was formed between them and the periblast.

Wilson (1891) was able to trace cleavages in the sea bass up to but not beyond the sixty-four-cell stage. Solberg (1939) in Fundulus could follow cleavage up to the sixty-four-cell stage, after which he made no attempt to count the blastomeres. Battle (1940) found it hard to count the blastomeres beyond the four-cell stage in Carassius; whereas for Salmo salar (Battle 1944) she was able to follow cleavages up to the thirty-two-cell stage. Carr (1942) in Micropterus salmoides observed the eight- and sixteen-cell stages and some thirty-two- and sixty-four-cell stages.

The Blastoderm

Cleavages continue rapidly so that by the end of the fourth hour of development the blastoderm is composed of several layers of hexagonal cells arranged in a mosaic pattern. Rapid segmentation is inferred from the fact that almost without exception the cells are in some phase of

division (fig. 11). All of the blastomeres possess a granular cytoplasm and some contain yolk granules. The surface cells of the four-hour blastoderm take a darker stain and are flattened dorsoventrally. These darkly staining cells later form the epidermal stratum and are somewhat separated from the deeper multicellular stratum beneath.

In blastoderms of six to eight hours of development, the mosaic pattern of arrangement is less pronounced and very few cells are in division as compared with the four-hour stage. The cells of the epidermal stratum thin out considerably and a definite separation between them and the deeper layer is discernable. Between the sixth and eighth hours of development the blastoderm undergoes certain changes in preparation for gastrulation. Sections of the blastoderm at this time reveal that it has become eccentrically thinned out anteriorly toward the definitive ventral lip of the blastopore and has become concave on its under surface. From all indications there is apparently a shifting of the blastomeres toward the future dorsal lip of the blastopore immediately above the oil globule. The significance of these changes will be discussed more fully under the topic of "Gastrulation."

The Periblast

Sections of the blastoderm, after four hours of development, show that the substance of the marginal blastomeres appears to be continuous with the surface protoplasm of the yolk, which is thicker around the edges and is thrown up into a ridge surrounding the periphery of the blastoderm (fig. 7, ppr.). This protoplasmic ridge is the forerunner of the periblast and has been designated by Wilson (1891) as the "pre-periblastic ridge."

The term "periblast" has been used by various authors to designate the syncytium which overlies the yolk during development. Others have used the same term as a designation for the protoplasm containing the nuclei. In order to avoid confusion, the term "periblast" as employed in this discussion applies not only to the protoplasm but to the nuclei as well.

In the largemouth black bass the periblastic nuclei appear to be derived only from the marginal cells of the blastoderm. Sections of blastoderms of four to six hours of development show that the marginal cells undergo horizontal cleavage and that the lower cells resulting from this division lose their cellular outlines and become incorporated within the protoplasm of the pre-periblastic ridge. The nuclei thus liberated are at first concentrated around the margin of the blastoderm in the pre-periblastic ridge, which now, according to Wilson (1891), should be called the periblastic ridge. These findings are in general agreement with those of Agassiz and Whitman (1884) and Wilson (1891) who found that the periblast is enlarged when cells at the periphery (of the blastoderm) lose their outlines and become part of the periblast. This loss of cellular outline has been described by various authors (Kupffer 1884, Henneguy 1888, Wilson 1891, and others) as manifesting itself in the formation of a "wreath" of lightly staining cells surrounding the blastoderm. Further agreement is found in the work of Carr (1942).

The presence of periblast nuclei in teleostean eggs was recognized as long ago as 1854 by Lereboullet. However, it was thirty years later, in 1884, that their origin was determined by Agassiz and Whitman in Ctenolabrus. According to these investigators, the periblastic nuclei were derived from two marginal rows of blastodermal cells. In the meantime, however, Hoffmann (1883) had advanced the theory that the

periblastic nuclei were derived from the first segmentation of the egg nucleus, which divided horizontally. The lowermost daughter cell produced from this division then passed into the yolk and became the progenitor of all the periblastic nuclei. Hoffmann's theory was severely criticized by Agassiz and Whitman (1884) and its inclusion here is only a matter of historical importance. The work of Wilson (1891) is largely a confirmation of that done by Agassiz and Whitman (1884) regarding the origin of the periblastic nuclei, and at the present time it is commonly believed that they have a blastodermic origin.

After the periblastic nuclei are formed they increase in number through mitotic division. Apparently these divisions take place only in the periblastic ridge and are completed prior to the beginning of periblast migration, for only in sections of the four-hour blastoderm and in the periblastic ridge can periblastic nuclei with division spindles be observed (fig. 12).

Multiplication of the periblastic nuclei is followed by migration and growth. The nuclei migrate underneath the blastoderm to form the central periblast. In sections of the blastoderm of six to eight hours of development the periblast extends to the center of the blastoderm and is concentrated in the region of the oil globule. This agrees with Carr (1942) who states that by the eighth hour the nuclei reach the center of the blastodisc. Although I made no direct observations on the migration of the periblast, it seems most likely that it is promoted by the changes in form undergone by the blastoderm during this period. Wilson (1891) reached similar conclusions regarding the migration of the periblast and its nuclei. He considered that this migration was a passive one and that the nuclei were carried along as the periblast flowed under the

blastoderm.

During the period of growth the periblastic nuclei lose their blastodermal characteristics and become very large and vesicular. This growth also is characterized by the formation of numerous pseudopodial protuberances on the nuclei and, although it is generally agreed that locomotion is a function of the cytoplasm, the possibility of an independent amoeboid migration of these highly specialized nuclei should not be overlooked.

Shortly before gastrulation, there is a concentration of the periblastic nuclei beneath the edge of the blastoderm which lies immediately above the oil globule. It is along this edge that gastrulation is initiated. As to the purpose of this concentration, one can only conjecture. Wilson (1891) could ascribe no true function to the periblastic nuclei but was in agreement with Hoffmann (1884) and Ziegler (1887), who thought that because of their uniform histological nature the periblastic nuclei must have some special physiological function such as changing the yolk into a form usable by the developing embryo. Others (Kupffer 1868 and Henneguy 1888) thought that the periblastic nuclei were already present in the periblastic protoplasm before cleavage and that they contributed to the formation of the blastoderm. Henneguy (1888), in spite of the results obtained by Agassiz and Whitmann in 1884, very carefully describes the "passing" of the periblastic nuclei out of the periblast into the blastoderm. Others (Kupffer 1884 and Gensch 1882) thought that the periblastic nuclei, in addition to being a source of blood cells, contributed to the formation of the alimentary canal. A later and more novel idea concerning the function of the periblast was advanced by Reinhard (1924), who believed it to be a source of the primordial germ

cells. Oppenheimer (1934, '36), working on Fundulus, came to the conclusion that the periblast must contain some substance that passes into the blastoderm to initiate gastrulation. Further light was thrown on its function by Devillers (1947), who removed the blastoderm of Salmo fario and cultured it in nutrient solutions and found that only enlarged cell masses called "hyper-blastulae" developed in the absence of the yolk and the periblast. Subsequent experiments indicated that there was a substance liberated from the periblast and the yolksac epithelium of older embryos which was capable of controlling embryonic differentiation. Although the experiments of Oppenheimer and others tend to show the presence of an organizer in the periblast, its function as a nutritive organ and diffusion medium should not be overlooked. On the basis of what I have been able to observe, the periblast transforms the solid yolk granules into liquid periblastic protoplasm. This transformation is characterized by a breaking up and a dissolution of the large yolk platelets. There is no direct contact between the yolk and the embryo save through the periblast, and yet the yolk is gradually used up during development without the benefit of intraintestinal digestion in the sense that it occurs in Amphibia. The relative volume of the periblast increases, whereas that of the yolk decreases, during development. It is then not unlikely that the periblast in a sense digests the yolk and that the embryo in turn absorbs the digested food material.

Gastrulation

The two distinct regions established as a result of the thinning out of the blastoderm are an eccentric thinner anterior portion and a thicker peripheral portion. This eccentric thinner portion of the blastoderm

has been designated as the extra-embryonic area (Wilson 1891, and others). At the beginning of gastrulation this area is three to four cell layers in thickness, but as invagination and epiboly proceed it becomes thinned out and increases in size as a result of being drawn around the yolk, preceded anteriorly and laterally by the advancing germ ring. The cells of this region ultimately become incorporated within the ectoderm of the yolksac. In a surface view of an early stage in the formation of the germ ring, the extra-embryonic area (extra-embryonic membrane of Rugh) appears as a somewhat clear, ill-defined circle surrounded by an opaque peripheral ridge, the germ ring (fig. 5). According to Wilson (1891) the eccentric thinner portion of the blastoderm overlies what commonly would be known as a subgerminal cavity, provided one existed. However, such a cavity does not exist in the large-mouth black bass. The posterior, embryonic portion (Wilson 1891) of the germ ring is thicker than its lateral and anterior portions, corresponds to the posterior pole of the embryo, is the site of initial invagination, and is the dorsal lip of the blastopore. By the eighth to ninth hours of development invagination has taken place in the region of the dorsal lip and the germ ring has begun to grow epibolically downwards over the yolk. During this overgrowth of the yolk by the germ ring, the dorsal lip remains stationary in position relative to the oil globule, epiboly being restricted to the ventral and lateral lips. As the germ ring advances over the yolk, invagination occurs all along the ring but particularly in the region of the dorsal lip. Midsagittal sections of the early gastrula show a tongue of cells extending a short distance beneath the ectoderm, displacing the yolk and forming a space between the blastoderm and the periblast (fig. 13). This is only a displacement space and has

no known phylogenetic or ontogenetic significance. The invaginated cell mass at the dorsal lip may, on the basis of what is derived from it, properly be termed the "mesentoderm." Some older workers (Wilson 1891, Cunningham 1885b, Ryder 1884, and others) have applied the term "hypoblast" to this invaginated mass.

By ten to eleven hours after fertilization the blastoderm has spread over the yolk as far as the equator (figs. 3 and 4). By twelve to fourteen hours of development the germ ring has advanced far beyond the equator and encloses a yolk plug composed primarily of the oil globule and a small portion of the yolk (fig. 14, yp.).

During the growth of the blastoderm around the yolk, the posterior portion of the germ ring becomes sharply differentiated from the rest of the blastoderm. This differentiated area, because of the shape it assumes early in development, has been designated as the embryonic shield and is composed of ectoderm and the underlying invaginated mesentoderm. In a surface view of a rather advanced stage of its development, the shield appears to extend craniad as a blunt projection into the extra-embryonic area (fig. 6, es.). As the anterior region of the blastoderm grows around the yolk, the extra-embryonic area is increased and the head end of the future embryo (he. of fig. 6) continues to grow in the same direction. Sagittal sections of the embryonic shield of a gastrula of twelve to fourteen hours of development show that the mesentoderm has grown forward beneath the ectoderm and extends almost to the anterior limit of the shield (fig. 15, med.). Here the mesentoderm ends in a slightly enlarged mass of cells which has been called the anterior mass (Wilson 1891). According to Wilson, this mass does not undergo the same differentiation as does the mesentoderm of the trunk and from all

appearances furnishes the mesoderm of the head. Text figures 1 and 2 represent several stages in the formation of the embryonic shield, particularly the cephalic growth of the mesentoderm.

The blastopore closes by the sixteenth to eighteenth hour of development and a conspicuous median thickening in the shield denotes the formation of the body of the embryo. The germ ring which has grown around the yolk sphere joins with the stationary dorsal lip to completely enclose the whole yolk mass. As a result of this union a large mass of undifferentiated tissue is formed and because most of the caudal region arises from it, it has been termed the caudal mass ("bourgeon caudale" of the French and "Schwanzknopse" of the German workers). Text figures 3 and 4 diagrammatically represent lateral and posterior views respectively of the advancing germ ring over the yolk plug to close the blastopore.

To Goette (1873) should be given the credit for first describing the gastrula of teleosts. He believed that gastrulation was the result of cell growth which resulted in the formation of a "Randwulst" and that the subsequent centripetal growth of the blastoderm formed the hypoblast (mesentoderm). In opposition to the idea of Goette, Oellacher (1873) denied that any invagination occurred but believed that the thicker portion of the germ ring (the embryonic anlage) split up into the ectoderm, mesoderm and endoderm. Ryder (1884-87) considered the gastrula to be formed through the inflection of the margin of the blastoderm, whereas Wilson (1891) believed that gastrulation was brought about through differential mitoses in the embryonic portion of the germ ring. Solberg (1936) for Fundulus and Carr (1942) for Micropterus reported that the gastrula is formed through an inturning of the blastoderm. Battle (1944),

Text Plate 1

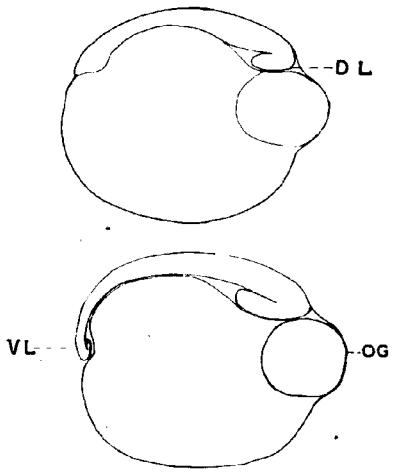
Explanation of Figures

1 Two stages in the formation of the embryonic shield.

2 Two stages in the formation of the embryonic shield, more advanced than those of figure 1.

3 Lateral view of the advancing germ ring over the yolkplug to close the blastopore. The dash-lines represent successive stages of this advance.

4 Posterior view of the advance of the germ ring to close the blastopore.



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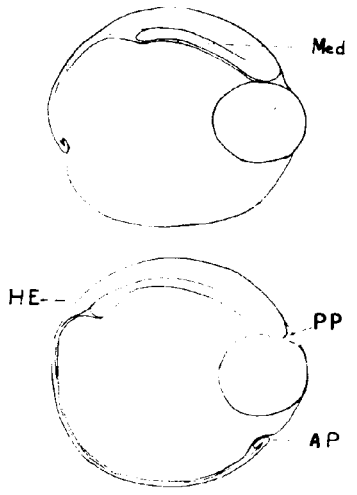

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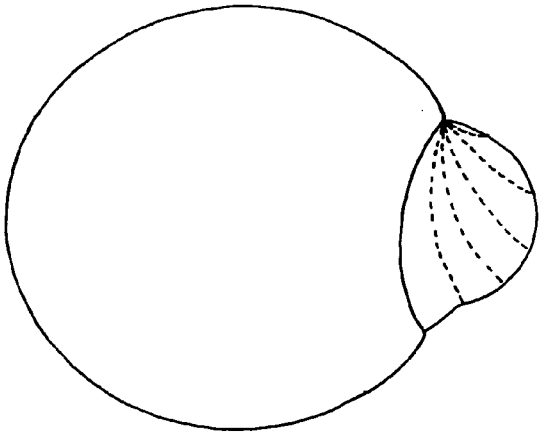

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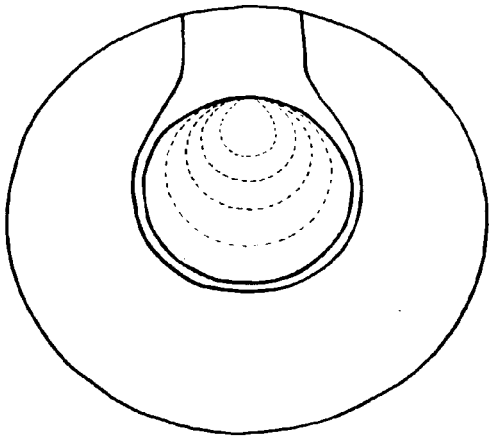


4

for the Atlantic salmon, reported that the two layered condition of the gastrula results from the formation of an elongated cleft in the embryonic shield which extends forward from the dorsal lip of the blastopore (delamination theory of Oellacher 1873). From my preparations it appears that gastrulation in the largemouth black bass is brought about through cell migration and not through differential mitoses. This interpretation is based on the fact that immediately preceeding and during gastrulation there is a conspicuous absence of mitotic figures in the blastoderm as a whole and particularly in the embryonic region of the germ ring. Text figure 5 shows diagrammatically the direction of cell movement during gastrulation.

Cell movement in the blastoderm of fishes was observed as long ago as 1874 by His. He observed this phenomenon during gastrulation and formation of the germ ring and subgerminal cavity in the blastoderm of the salmon and the trout. He believed that in these forms the eccentric flattening and thinning of the blastoderm was the result of an active peripheral migration of the loose internal blastomeres beneath the epidermal stratum. Recent experiments employing vital staining techniques have been made by Pasteels (1933, 1934, 1936 and 1937) on Salmo and by Oppenheimer (1935, 1936) on Fundulus. The results of these experiments prove beyond doubt that gastrulation and embryo formation are brought about through cell movements. Further experiments by various authors (Hoadley 1928, Lewis 1912, Luther 1935, 1936, and 1937, and others), in which deficiences in various portions of the embryo were produced, substantiate these results and lend additional credence to the theory of cell movement and relocation. Pasteels (1934 and 1936), after making numerous mitotic counts, was certain that in Salmo the activities related

to gastrulation were confined to cellular movement and rearrangement.

Germ Layer Formation

The Ectoderm. The ectoderm arises directly from the blastoderm and is composed of those blastomeres that do not invaginate. It consists of two distinct portions, the epidermal stratum and the nervous layer. The origin of the epidermal stratum (the "Deckschicht" of the German workers) from the surface cells of the blastoderm has already been described. It persists as a thin sheet of flattened cells covering the entire embryo and gives rise to the epidermal layer of the skin. Its structure in the early embryo is shown in figures 23 and 24. I found, as did Wilson (1891), that although there is a tendency for this layer to invaginate, it actually takes no part in this process (fig. 18). The second portion of the ectoderm, the nervous layer, consists of several strata of cells and is restricted to the embryonic shield. Early in shield formation it is thicker laterally than medially (fig. 19), and is consumed in the formation of the central nervous system, lateral line organs, and other special sense organs on the head (eye, lens, ear, nasal pits, and head canals). Sections of the closing blastopore show that the nervous ectoderm is continuous with the underlying mesentoderm in the neurenteric streak, an area which is homologous with the neurenteric canal (fig. 18).

The Mesentoderm. The mesentoderm is formed by invagination of the blastoderm, and from it arise the mesoderm and entoderm and their derivatives. Since the notochord does not differentiate as the mesentoderm invaginates, but is formed later from its axial portion, the term chorda-mesentoderm is not appropriate. The organization and

differentiation of the mesentoderm is best seen in cross sections of the embryo and in early stages of development (twelve to fourteen hours) when it is only two to three cell layers in thickness (fig. 19). The first differentiation of the mesentoderm is seen in the formation of the notochordal anlage from its axial portion (fig. 19).

The Mesoderm. Coincident with the formation of the notochordal anlage, the paraxial portion of the mesentoderm differentiates into mesoderm and entoderm. The mesoderm is formed from the upper layers of the paraxial portion and in embryos of twelve to fourteen hours consists of two lateral masses, the somite primordia, on each side of the developing notochord (fig. 19). In embryos of sixteen to eighteen hours the notochord is beginning to round up and the axial portions of the somites are thicker (figs. 20 and 23). By twenty to twenty-two hours, the somites have begun to extend dorsally (fig. 21) and by thirty to thirty-two hours are divided by a horizontal constriction or septum into epiaxial and hypaxial muscle divisions (fig. 22). As the somites thicken axially, the nerve cord makes its appearance in the form of a blunt wedge resting on the notochord. This wedge appears to result from a median shifting of the nervous ectoderm (figs. 19, 20 and 21). As the organization of the embryo progresses the mesodermal somites differentiate into two distinct areas - the muscle plates, already described, and the lateral plate or nephridiocoelomic mesoderm. Text figure 9 illustrates diagrammatically several stages in the differentiation of the somite and the formation of the medullary wedge.

The Entoderm. The entoderm is formed by a process of delamination from the paraxial mesentoderm. At first it is discontinuous beneath the

Text Plate 2

Explanation of Figures

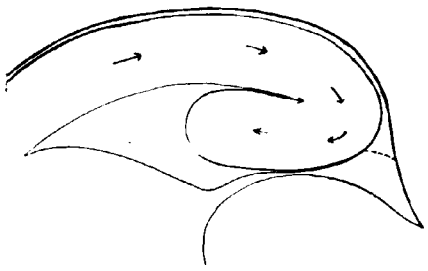
5 A diagram showing the direction of cell movement during gastrulation and formation of the embryonic shield.

6 A diagram showing the relationship of the dorsal lip excrescence of potential germ cells. The germ cells are represented by the dark circles and are shown passing from the excrescence into the periblast ventral to the dorsal lip of the blastopore.

7 A diagram showing an early relationship of the germ cells to the caudal yolksac extension. The dash-line represents the definitive position of the gut.

8 A diagram showing a later relationship of the germ cells to the caudal yolksac extension. The germ cells are shown passing out of the extension into the mesoderm ventral to the gut and then dorsad to the dorsal mesentery. The dash-lines represent the right pronephric duct. The pronephric ducts are shown entering the gut near the anus. The subintestinal vein is shown passing between the pronephric ducts and sinistroventrad around the gut and continuing anteriad as the vitelline vein beneath the yolk.

9 A diagrammatic representation of several stages in the differentiation of the somite and the formation of the medullary wedge. The arrows indicate the direction of cell movement.



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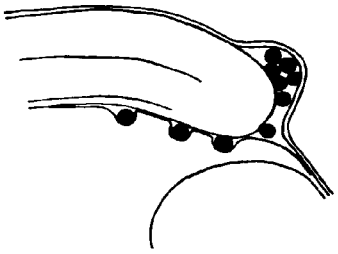

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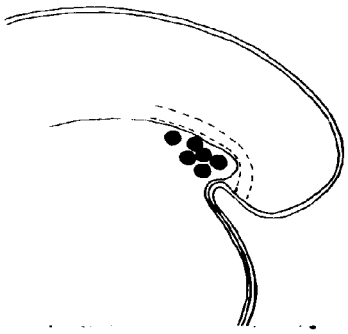

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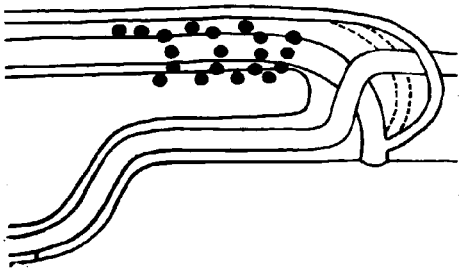

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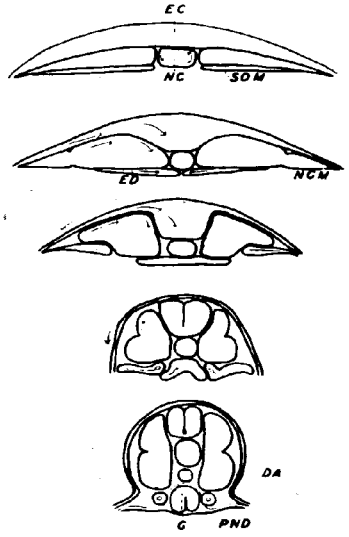

9

notochord but later in development it grows mesiad to form a continuous layer. In a sixteen to eighteen hour embryo, the entoderm consists of two lateral lamellae only one cell in thickness. At times these lamellae are separate and at other times they appear to be continuous beneath the notochord. The entoderm, like the mesoderm and nervous ectoderm, is restricted to the embryonic shield (fig. 23) and, contrary to the general conception of a yolksac, it does not continue around the yolk as a yolk-sac entoderm. The periblast apparently takes over the function of enclosing the yolk and in a broad sense might be regarded as homologous with the yolksac entoderm of higher forms. Thus at one time in its development, before the ventral growth of the hypomeric mesoderm, the yolksac of Micropterus consists of a layer of ectodermal cells, the epidermal stratum, and the periblast covering the yolk. The entoderm will be discussed further under the topic of "Gut Formation."

Formation of the Gut and Kupffer's Vesicle

Gut Formation. By the twenty-second hour of development the entoderm from each side has grown mesiad beneath the notochord to form a continuous layer. In the trunk region the gut is formed through a folding along the midline of the entodermal lamella; whereas in the branchial region two lateral branchial folds are produced which grow both mesiad and laterad to join each other and also the ectoderm at each side. Gut formation begins first in the region of the so called Kupffer's vesicle (discussed later), and a short time later in the branchial region. In the branchial and trunk regions all of the entoderm is used up in the formation of the gut. In the region of Kupffer's vesicle, however, some lateral undifferentiated entoderm remains. Gut formation as it occurs

in the trunk region is diagrammatically represented in figure 8. Although a similar arrangement of cells as shown in this figure might not exist for any two embryos, for the sake of brevity in description only nine cells have been represented in it. The arrows indicate the direction of cell movement. Using the diagrams as a basis, gut formation may be described as follows: The inner margins of cells number two grow axiad and in so doing cause the "keystone cell", number one, to lift upward, thus establishing a small cavity between it and the periblast. Cells number three, grow mesiad and slip under cells number two. Cells number four follow their predecessors and slip under cells number three. The tube is completed when cells number five come together. These findings agree with and for the most part confirm those of Wilson (1891), Solberg (1939) and Battle (1944). They agree only in part with those of Ziegler (1882) and Henneguy (1888) and are contrary to those of Kingsley and Conn (1883), Agassiz and Whitman (1884), Cunningham (1885) and Ryder (1887).

Kupffer's Vesicle. In embryos of twenty-two hours of development Kupffer's vesicle is formed and lies between the caudal mass and the periblast. The vesicle forms in the same manner as the gut and becomes the definitive cloacal region of the alimentary tract. In the beginning its floor is the periblast as is also that of the gut. It is converted into a tube by the fusion of its lateral walls. The vesicle lies anterior to the neurenteric streak but is not continuous anteriorly with a certain mass of entodermal cells as reported by Wilson (1891) for Serranus. The vesicle was not observed to atrophy nor to form any structure that would correspond to a solid postanal gut.

The vesicle, named after Von Kupffer who first described it in 1868, has received more attention in literature than its importance warrants.

Various investigators who have studied the embryology of fishes have offered theories and comments as to its origin and fate. Von Kupffer thought it to be an invagination from the ectoderm and thus to be homologous with the ectodermal vesicle described for reptiles. Cunningham (1885) believed it to be the terminal part of the archenteron and Wilson (1891) interpreted it as being homologous with the postanal vesicle in selachians. Others have related it to the cloaca.

My findings agree with those of Henneguy (1888), who says: "La vesicle de Kupffer n'est donc que la premier apparition de la cavité du tube digestif avec laquelle elle se confond plus tard." (p. 563).

Considerable controversy has been waged as to whether or not a cellular floor is present in the vesicle from the beginning. Kingsley and Conn (1883), Agassiz and Whitman (1884), Cunningham (1885) and Wilson (1891) state that the vesicle arises as a space between the periblast and the entoderm. Contrary to this, Henneguy (1888) and Schwarz (1889) assert that it arises in the caudal mass and that from its inception it has a cellular floor. My findings agree with those of Wilson (1891) and others.

Formation of the Coelom and Kidney Ducts

Coelom Formation. The coelom takes its origin from the lateral portion of the nephridiocoelomic mesoderm, whose mesial portion enters into the formation of the kidney ducts. After the dorsal evagination and constriction of the pronephric ducts, the remaining mass of hypomeric mesoderm grows around the gut, mesiad to it and the ducts, to form the broad dorsal and ventral mesenteries. Careful observation reveals that the mass is composed of two layers of cells closely applied to one

another with no visible space between them. These layers separate later to form the coelom. The coelom first makes its appearance in the anterior trunk region simultaneously with the formation of the pronephric ducts. In the midtrunk region of a forty-seven hour embryo the coelomic layers extend a short distance lateral to the gut. As development progresses, the layers grow ventrad between the ectoderm and the yolk to fuse in the midventral line and completely enclose the yolk within the ventral mesentery. A diagrammatic representation of the ventral growth of the coelomic layers is presented in figure 9. A cavity appears somewhat later in development between the splanchnic and somatic layers. In the region of the hindgut of a 3.5 millimeter larva, the appearance of a cavity at the dorsal mesenteric angle marks the beginning of the coelom posteriorly (fig. 43). The somatic layer of mesoderm is widely separated from the ectoderm by a large lymph space. As larval growth continues, this space is gradually reduced and finally disappears, leaving the ectoderm and somatic mesoderm in juxtaposition. By the time the larva reaches 5.0 millimeters in length, a typical coelom lined with a delicate mesothelium has formed (fig. 46). The lymph spaces present in the 3.5 millimeter larva have been obliterated and the gut is bounded by coelomic spaces on either side.

Kidney Duct Formation. In the largemouth black bass the kidney ducts are formed from the somatic layer of the coelom by a process of evagination and constriction. Formation of the ducts begins in the anterior portion of the embryo and proceeds posteriorly. In its anterior portion the duct has a cavity from the beginning, while in its posterior portion it is solid and composed of cells arranged in a radial fashion. The cavity in the anterior portion of the duct is formed from the

coelomic cavity as the duct is evaginated and constricted off. Sections taken through the anterior trunk region of thirty-four to thirty-six hour embryos show that there is a dorsal evagination of the somatic layer of the coelom at its axial angle near the gut. This evagination contains a very minute cavity, which is continuous with that of the coelom. When traced forward the cavity ends blindly in the tubule, and it appears that there is no anterior opening of the duct through a nephrostome into the coelom. At no time during the formation of the ducts do they possess a cavity throughout their entire length. In the anterior and middle trunk region they lie lateral to the gut and in the posterior region they lie dorsal and dorsolateral to it. There is a transitory connection of the ducts posteriorly with the cloaca, but later in development this connection is lost and the ducts empty to the outside by their own opening in the urinogenital papilla. The pronephric ducts persist as the functional excretory ducts of the mesonephros and at no time do they enter into the formation of the reproductive system.

These findings are in agreement with those of Wilson (1891), Swaen and Brachet (1899) and others regarding the origin of the ducts, but they are contrary to the findings of Wilson (1891) and Carr (1942) regarding the presence of an anterior opening of the tubule into the coelom.

The Subintestinal Yolksac Extension

Commensurate with the caudal growth of the embryo, a caudal extension of the yolksac is carried back by the tail. This extension lies ventral to the gut and dorsal to the subintestinal vein and is surrounded by the ventral mesentery. Its contents consist primarily of liquid periblastic yolk. In a forty-four hour embryo the extension is quite

large relatively as compared to the size of the gut. A section through the yolksac extension of an embryo of this age serves to illustrate its relative size and position (fig. 30). In this section the yolk is not shown because it was withdrawn purposely prior to fixation and only the space that it once occupied remains. The size of this caudal extension in relation to the gut and other associated structures, and also the fact that the extension is bounded by the ventral mesentery, should be noted. The subintestinal vein lies in the mesentery where the two mesenteric layers meet along the ventral midline (fig. 30). Anteriorly, the extension is continuous with the yolksac, and posteriorly it ends blindly in the mesentery. As larval development progresses the relative size of the extension is reduced. In the 3.5 millimeter larva it is comparable in size to the gut (fig. 36), and in the 4.5-5.5 millimeter larva it is all but obliterated. Text figure 8 also shows diagrammatically the relationships of the gut, the yolksac extension, and the subintestinal vein.

PART II HISTORY OF THE GERM CELLS

Historical Résumé

1. Vertebrates in General

Three theories concerning the origin of the germ cells in vertebrates have been advanced. The earliest of these is the "germinal epithelium theory" proposed by Waldeyer in 1870. According to this theory, the germ cells, or "Ureier," take their origin in situ in the thick coelomic epithelium covering the gonad and are consequently considered to be of epithelial or mesodermal origin. This theory of origin for the germ cells was generally accepted until challenged a decade later by Nussbaum (1880). This investigator, while studying the germ cell history of the trout and other species, concluded that the germ cells originate extragonadally and later migrate to the germinal epithelium of the gonad, where Waldeyer (1870) first saw them. On the basis of his investigations, Nussbaum advanced the theory of an "early segregation" of the germ cells, with the implication that their progenitors were of extragonadal origin and were derived from cells segregated early in development. Thus, with the advent of two diametrically opposed theories of germ cell origin and with the appearance in 1886 of Weismann's hypothesis of germ cell continuity, interest in this problem received considerable stimulation.

A few years later, a third theory was proposed by Rückert (1888), which has since become known as the "gonotome theory." According to it, the germ cells originate in segmental mesodermal elements of the lateral plate, called gonotomes, to correspond in terminology with other mesodermal structures of similar arrangement. The theory is based on an

attempt at homology with the segmental gonads of amphioxus. This concept was further elaborated by Van Wijhe (1889) in his work on Pristiurus and later by Hall (1904) and Dustin (1907) and others in investigations of the amphibian genera Ambystoma, Triton, Bufo, Rana and others.

From a review of the literature dealing with the germ cells and their history in the vertebrates it is evident that considerable disagreement exists as to their origin, mode of migration, marks of identification, and ultimate fate. It is not uncommon to find two competent investigators working with the same material arriving at contrary conclusions. Heys (1931) and Everett (1945) have summarized the various existing ideas, and according to them there are four groups or schools of thought on the subject: (1) those who do not accept the idea of an early segregation of the germ cells (Hargitt, Simkins, Von Berenberg-Gossler and others); (2) those who admit an early segregation of the germ cells but believe that these degenerate and do not give rise to definitive reproductive cells (Felix, B. Allen, Firket, Kingery and others); (3) those who believe that the germ cells arise both from the soma and from primordial germ cells (Humphrey, Brambell, B. Allen, Becarri, Burns, McCosh and others; and (4) those who believe that the early segregated cells are the only source of definitive sex cells (Nussbaum, Okkelberg, Beard, Eigenmann, Witschi, Burns, Cheng, Blocker, Everett and others).

It is interesting to note that very few of the investigations considered by Heys and Everett in their reviews of the status of the germ cell problem in vertebrates dealt with the germ cell history in fishes. It goes without saying, however, that in such a large vertebrate group as this, marked differences in opinions, conclusions and results may be

found. In this group, as in most others, the influence of Waldeyer was reflected in the findings of the earlier workers. Most of the recent workers, however, support the idea of an early segregation of the germ cells. There are, nevertheless, a few who adhere to the idea of a partial degeneration of the primordial cells or a dual origin for the ova, while certain others hold to a wholesale disintegration of the so called primordial germ cells and believe that the definitive sexual elements are of stromal or epithelial origin.

The following table is an extended summary of literature on the subject which has appeared since the publications of Okkelberg (1921), Cheng (1932), and Blocker (1933). The attempt is not made to present all the literature but to give a representative sample of that which has been done.

Table 1

Summary of Some of the Literature Dealing with the Origin of Germ Cells in Vertebrates

Author	Date	Animal*	Remarks on Germ Cells
		CYCLOSTOMES	
Goette	1890	Petromyzon fluviatilis	Germ cells come from mesoderm.
Wheeler	1899	Petromyzon planeri	Germ cells from blastoderm.
Beard	1902	Petromyzon planeri	Germ cells are early segregated cells.
Okkelberg	1921	Entosphenus wilderi	Definitive germ cells from early segregated cells.

*Technical names are those given by authors although for some of the animals other names are now used.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
Butcher	1929	Petromyzon marinus unicolor	Dual origin of germ cells: (a) from early segregated cells; (b) from coelomic epithelium.
		ELASMOBRANCHS	
Semper	1875	Plagiostomes	Germ cells from coelomic epithelium.
Balfour	1876 1877	Scyllium Pristiurus	Germ cells probably from mesoderm.
Rückert	1888	Pristiurus	Germ cells from gonotome.
Van Wijhe	1889	Scyllium Pristiurus	Germ cells from gonotome.
Rabl	1896	Pristiurus	Germ cells found extra-regionally.
Beard	1900 1902	Raja batis Pristiurus	Germ cells from early segregated cells.
Woods	1902	Squalus acanthias	Germ cells first seen in entoderm.
		ACTINOPTERYGIANS (Berg 1940)	
Allen	1911	Amia Lepisosteus	Germ cells from early segregated cells.
Nussbaum	1880	Trout	Germ cells from early segregated cells, not derived from mesoderm.
MacLeod	1881	Hippocampus Belone	Germ cells from germinal epithelium.
Hoffmann	1886	Salmon	Germ cells from peritoneum.
Eigenmann	1891	Micrometrus aggregatus	Germ cells early segmentation cells of 5th. or 6th. cleavage of the egg.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
Böhi	1904	Trout Salmon	Germ cells from germinal epithelium.
Fedorow	1907	Salmo fario	Germ cells in somatopleure and splanchnopleure.
Dodds	1910	Lophius piscatorius	Germ cells are early segregated cells.
Bachmann	1914	Amiurus nebulosus	Germ cells from early segregated cells.
Richards and Thompson	1921	Fundulus heteroclitus	Germ cells are early segregated cells.
Essenberg	1923	Xiphophorus helleri	Female: germ cells from ovarian cortex and epithelium of ovary. Male: from the epithelium of tubules.
Reinhard	1924	Scardinius	Germ cells from giant cells of periblast.
Van Oordt	1924	Xiphophorus helleri	Male: germ cells from epithelium of tubules.
Hann	1927	Cottus bairdii	Germ cells from giant entodermal cells.
Foley	1927	Umbra limi (male)	Spermatogonia from stromal cells of testis.
Wolf	1931	Platypoecilus maculatus	Male: spermatogonia from primordial cells. Female: ova from primordial cells and germinal epithelium.
Goodrich et al	1934	Lebistes reticulatus	Germ cells are early segregated cells first seen in mesentoderm.
Maschkowziff	1934	Acipenser stellatus Salmo trutta	Primordial germ cells found in gut entoderm. Dimorphic from very beginning.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
		ACTINOPTERYGIANS (cont.)	
Bennington	1936	Betta splendens	Adult males: germ cells from residual cells-descendents of primordial germ cells.
Dildine	1936	Lebistes reticulatus	Germ cells seen in mesentoderm soon after gastrulation.
Odum	1936	Opsanus tau	Germ cells occur in two strands in post coelomic region.
Moore	1937	Salmo irideus	Germ cells are early segregation cells.
		AMPHIBIANS	
Nussbaum	1880	Rana fusca	Germ cells are early segregated cells.
Hoffmann	1886	Triton, Rana Bufo	Germ cells from germinal epithelium.
Semon	1891	Ichthyophis glutinosus	Germ cells from the germinal epithelium.
Bouin	1900 1901	Rana temporaria	Germ cells from early segregated cells and from peritoneal and mesenchymal cells.
Hall	1904	Ambystoma punctatum	Germ cells from a gonotome.
Allen	1907	Rana pipiens	Germ cells from early segregated cells, first seen in entoderm.
Dustin	1907	Triton alpestris Rana fusca Bufo vulgaris	Germ cells from the gonotome and from peritoneal cells.
King	1908	Bufo lentiginosus	Germ cells originate in entoderm, migrate into the mesoderm.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
		AMPHIBIANS (cont.)	
Kauschake-witsch	1910	Rana esculenta	Primary germ cells from early segregated cells, functional germ cells from mesenchyme and peritoneum.
Allen	1911	Ambystoma Necturus	Germ cells from mesoderm lying between myotome and lateral plate.
Schapitz	1912	Ambystoma mexicanum	Germ cells from the gonotome.
Spehl and Polus	1912	Ambystoma tigrinum	Germ cells from gonotome and peritoneal cells.
Abramowicz	1913	Triton taeniatus	Primary germ cells from entoderm; secondary from mesoderm.
Champy	1913	Triton palmatus Rana temporaria	Germ cells from gonotome.
Witschi	1914	Rana temporaria	Germ cells first found in entoderm.
Gatenby	1916	Rana temporaria	Germ cells of adults originate seasonally from peritoneal cells.
Swingle	1921	Rana catesbeiana	Primordial germ cells from entoderm. Secondary cells from descendents of primary cells.
Becarri	1922	Salamandrina perspicillata	Germ cells from mesoderm (gonotome).
Hargitt	1924	Diemyctylus viridescens	Germ cells derived from epithelium of collecting ducts and germinal epithelium.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
		AMPHIBIANS (cont.)	
Bounoure	1924 1925	Triton alpestris Rana temporaria Bufo vulgaris	Germ cells first found in entoderm.
Obreshkove	1924	Diemyctylus viridescens	Strong possibility of stromal transition into germ cells.
Humphrey	1925 1925	Rana pipiens (and others) Triturus viridescens (and others)	Germ cells appear first in entoderm. Germ cells appear first in medial part of lateral plate mesoderm.
Burns	1925	Ambystoma punctatum	Germ cells are early segregated cells.
Perle	1927	Bufo vulgaris	Germ cells located first in entoderm.
Witschi	1929	Rana sylvatica	Germ cells originate in entoderm.
Christensen	1930	Rana pipiens	Germ cells seen in entoderm.
Chen	1930	Necturus maculosus	Germ cells from mesoderm.
McCosh	1930	Ambystoma maculatum	Germ cells from lateral mesoderm - somatic in origin.
Cheng	1932	Rana catabrigensis	Germ cells are early segregation cells.
Bounoure	1934	Rana temporaria	Germ cells determined by presence of a "cytoplasm germinale" first located at posterior pole of egg.
Fischer	1935	Axolotl	Germ cells from primary gonocytes (germ cells).
Burger	1937	Plethodon cinereus	Primordial germ cells give rise to definitive sex cells.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
		AMPHIBIANS (cont.)	
Seshachar	1937	Ichthyophis glutinosus	No spermatogonia traceable to primordial germ cells; arise from epithelium of collecting tubules.
Nieuwkoop	1946	Triton and Ambystoma mexicanum	Germ cells differentiate in the lateral plate of presumptive mesoderm.
		REPTILES	
Allen	1906 1907 1911	Chrysemys marginata	Germ cells are early segregated cells first seen in the entoderm.
Jarvis	1908	Phrynosoma cornutum	Germ cells from early segregated cells appear first in the entoderm.
Dustin	1910	Chrysemys marginata	Primary germ cells from entoderm. Secondary from epithelial cells.
Von Berenberg -Gossler	1914	Lacerta agilis	Germ cells from mesodermal cells. So called primordial cells are misnamed.
Jordon	1917	Caretta caretta	Primordial germ cells recognized in yolksac epithelium.
Simkins	1925	Trionyx	No evidence to show an extraembryonic origin for the primordial germ cells.
Risley	1933	Sternotherus odoratus	Germ cells from early differentiated cells.
	1934	Sternotherus odoratus	Definitive germ cells from primordial germ cells and from coelomic epithelium.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
		BIRDS	
Waldeyer	1870	Chick	Ova from germinal epithelium, spermatogonia derived from epithelium of Wolffian duct.
Hoffmann	1892	12 species	Germ cells are early segregated cells.
Nussbaum	1901	Chick	Germ cells first seen in splanchnopleure.
Rubaschkin	1907	Chick Duck	Germ cells are early segregated cells.
Tschaschin	1910	Chick	Germ cells are early segregated cells.
Von Berenberg -Gossler	1912	Chick	So called primordial cells seen in splanchnopleure are not germ cells.
Swift	1914 1916	Chick	Germ cells first seen in proamnionic region are entoderm in origin.
Firket	1914 1920	Chick	Germ cells primarily from germinal epithelium possibly from primordial germ cells.
Goldsmith	1928	Chick	Germ cells arise in an extraembryonic position and give rise to definitive sexual elements.
Dantschakoff	1931	Chick	Primordial germ cells from entodermal wandering cells.
Matsumoto	1932	Chick	Primordial germ cells from early segregated cells.
Blocker	1933	Sparrow	Germ cells are early segregated cells first recognized in proamnion.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
		BIRDS	
Witschi	1935	Sparrow	Germ cells first seen in yolksac splanchnopleure.
		MAMMALS	
Allen	1904	Pig Rabbit	Functional germ cells from peritoneal cells.
Rubaschkin	1908 1909 1912	Cat, Rabbit Mole Guinea pig Porpoise	Early segregation of primordial germ cells; located in entoderm and give rise to definitive sex cells.
Sainmont	1906	Cat	Primordial germ cells (ova) present but not functional. Functional cells arise from epithelium of peritoneum.
Winiwarter and Sainmont	1909	Cat	Definitive germ cells from germinal epithelium.
Kirkham	1916	Mouse	Oogonia from primordial cells, spermatogonia from epithelial cells.
Vannemann	1917	Armadillo	Germ cells first seen in entoderm of blastocyst.
Kingery	1917	Mouse	Ova from germinal epithelium.
Firket	1920	Chick Albino rat	Early primordial germ cells degenerate, not progenitors of definitive sex cells.
Hargitt	1925	Rat	Germ cells from germinal epithelium.
Cowperthwaite	1925	Rat	No origin of germ cells from germinal epithelium.

Table 1 cont.

Author	Date	Animal	Remarks on Germ Cells
		MAMMALS (cont.)	
Butcher	1927	White rat	Primordial germ cells degenerate, definitive sex cells from germinal epithelium
Brambell	1927	Mouse	Ova from germinal epithelium.
Heys	1931	Albino rat	Germ cells are early segregated cells.
da Costa	1932	Guinea pig	Primordial germ cells first seen in allantoic mesoblast.
Politzer	1933	Man	Germ cells first seen in yolksac entoderm of presomite embryo.
Hamlett	1935	Man	Germ cells occur in mid-gut, not found elsewhere.
Bookhout	1937	Guinea pig	Early primordial cells degenerate, not ancestors of definitive sex cells.
Trabucco	1938	Rabbit	Germ cells located in 7 day embryo. Early differentiated cells.
Kingsbury	1938	Cat	No functional ova from germinal epithelium, all from primordial germ cells.
Everett	1942	Opossum	Germ cells from germinal epithelium.
Everett	1943	Mouse	Primordial germ cells are set aside early in development, first seen in entoderm.
Witschi	1948	Man	Primordial sex cells first found in yolksac and allantoic extension of yolksac.

2. Fishes

Our discussion will be confined principally to the problem as it concerns the history of the germ cells in fishes. The brief historical account will be followed by my own findings in the largemouth black bass.

A. Cyclostomes. One of the first accounts dealing with the germ cell history in cyclostomes was that of Goette (1890). This investigator thought that the germ cells in Petromyzon fluviatilis were mesodermal in origin. The works of Wheeler (1899) on Petromyzon planeri, Beard (1902) on Petromyzon planeri, and Okkelberg (1921) on Entosphenus wilderi (Gage) all support an early segregation of the germ cells from blastoderm cells. Butcher (1929), however, presented evidence that the germ cells in Petromyzon marinus unicolor have a dual origin: namely, from early segregated cells and from the coelomic mesoderm.

B. Elasmobranchs. Semper (1875) believed that in plagiostomes the germ cells arose in the coelomic epithelium covering the gonad. Balfour (1876, 1877), working with Scyllium and Pristiurus, concluded that the germ cells probably had their origin in the mesoderm. Rückert (1888) and Van Wijhe (1889), however, also working on Scyllium and Pristiurus, theorized that the germ cells arose from a portion of the segmental mesoderm called the gonotome. Beard (1900), after investigating Raja batis and Pristiurus (1902), reported that the germ cells in these forms arose from early segregated cells. Woods (1902) first recognized the germ cells of Squalus acanthias in the entoderm.

C. Actinopterygians. The only complete work on the germ cells of more primitive members of this group is that of Allen (1911), who found that in Amia and Lepisosteus the germ cells could be recognized in early stages of development in the entoderm of the subgerminal cavity near the

region of the hindgut. He described them as being very large cells containing many yolk granules and capable of independent movement. He found that in Amia practically all the germ cells reach the gonadal primordia while in Lepisosteus only about half of them reach their destination. In addition, he concluded that in both forms the number of primordial germ cells is subject to individual variation.

The initial study of the germ cells in higher members of this group, that of Nussbaum (1880) on the trout which resulted in the advancement of the early segregation theory and its incorporation in the germ cell continuity hypothesis of Weismann in 1886, has already been mentioned. Another of the earlier studies is that of Eigenmann (1891) on Micrometrus aggregatus. According to him the primordial germ cells were segregated as early as the fifth or sixth segmentation. However, he states that, "... the sex cells can first be distinguished from the surrounding cells about the time the blastopore closes. ...[in eggs]in which the mesoderm is not yet split off from the entoderm" (p. 483).

Böhi (1904) believed that the germ cells in the salmon and trout originated in the germinal epithelium and that they were of mesodermal origin.

Fedorow (1907), working on Salmo fario, observed the germ cells in the somatopleure and splanchnopleure of the eighteen-day embryo. Most of them were in the somatopleure and became lodged there when the splanchnic and somatic layers separated from one another.

Dodds (1910) found that in Lophius piscatorius the primordial germ cells could be recognized in the primary entoderm before the formation of the embryo proper: "... at a stage when the blastoderm has not quite half covered the yolk and the formation of the embryo has but begun"

(p. 594). He found that the primordial germ cells could be recognized by the presence of an extruded plasmosomal body and that at one time all of them were located in the myotome and migrated through it. In addition he found that the primordial germ cells do not divide during their migration prior to reaching the gonad region and that their migration was in part active and in part passive.

Sink (1912) found that in Opsanus tau the germ cells first could be recognized in the gut entoderm and many were in the process of mitosis. Later, they migrated from the entoderm through the mesentery to the germ ridges ventral to the pronephric ducts where they became about evenly distributed between the two sex glands. He recognized them by their large size, circular outline and well defined nucleus and nucleolus.

The origin and migration of the germ cells in Amiurus nebulosus were studied by Bachmann (1914). She found that they were first recognizable in the margins of the lateral plate as amoeboid cells containing yolk granules and possessing peculiar staining capacities. In young embryos the germ cells were located in the mesoderm near the region of the closing blastopore, and in embryos 3.2 to 3.7 millimeters long, they appeared in the mesoderm anterior to the point of attachment of the tail to the body. She found no particular characteristic which could be used to identify the primordial germ cells such as Dodds had found for Lophius, nor did she observe any transition of peritoneal cells into germ cells.

Richards and Thompson (1921), in their study of the sex cells of Fundulus heteroclitus, found that the earliest stage in which the germ cells could be recognized was when the germ ring had just closed and the tail was just beginning to elongate. At this time the sex cells were located in the extra-embryonic region lateral to the undifferentiated

entodermal cell mass of the posterior half of the embryo. According to them, the migration of the germ cells is passive, resulting from the normal processes of growth. They believed that the germinal path led from the peripheral entoderm into the mass of undifferentiated entodermal cells. When this cell mass split to form gut entoderm and lateral mesoderm, the sex cells proceeded mesiad with either the somatic or splanchnic layer. By the time the gut is formed the primordial cells are lateral to it, and eventually they become located in the splanchnic mesoderm. From here they migrate dorsad around the hindgut, then to the region ventral to the Wolffian ducts. These workers described the sex cells of Fundulus as possessing a lightly staining cytoplasm, a linin system connected with two centrally located nucleoli, and a peripheral arrangement of the chromatin in the nucleus, the last characteristic being a constant distinguishing feature. A large centrosome usually was present in the cytoplasm, even when the cells did not appear to be preparing to divide.

Essenberg (1923), studying sex differentiation in Xiphophorus helleri Heckel, found that the primordial germ cells were the largest cells in the body of the fish, having an average diameter of 14.4 micra with a nucleus of 8.4 micra in diameter. He found that the nucleus took a lighter stain than the cytoplasm, possessed one or more distinct nucleoli, and contained chromatin arranged in loops and strands immediately adjacent to the nuclear membrane. He reported that the ovary in fishes of a certain length underwent regression and the primordial germ cells appeared to disintegrate. He believed that in the female the definitive germ cells originated later from peritoneal cells and from "free" cells in the ovarian cortex, and that in the male the definitive germ cells originated from peritoneal cells. However, the fate of the primordial cells in the male was uncertain.

Van Oordt (1924), working on the same species, reached the same conclusions regarding the origin of the definitive sex cells.

Reinhard (1924) found that in Scardinius the germ cells arose from giant cells of the periblast. He termed these cells "Riesenzellen" and thought that they originated from periblast nuclei and that they migrated into the mesoderm by amoeboid movement.

Giant cells also were observed by Hann (1927) in Cottus bairdii Girard. He found no evidence that they came from periblast nuclei and considered them to be entodermal in origin. He found that the primordial germ cells were derived from these giant cells and that they gave rise to the definitive sex cells of both sexes. Although he does not indicate how the germ cells migrate, from his description it appears that this migration was passive, resulting from the shifting of the gut ventrad.

Foley (1927), studying the spermatogenesis in the male mud minnow, Umbri limi, concluded that the spermatogonia were derived from stromal cells of the testis and not from the primordial germ cells.

Wolf (1931) concluded from his studies on Platypoecilus maculatus that the definitive ova have a dual origin: mainly from the primordial germ cells, and to a lesser degree from the germinal epithelium. However, he believed that the spermatozoa arose only from the primordial germ cells. He described the primordial germ cells as being amoeboid, that their migration from the periphery of the lateral plate to the lateral aspects of the gut was active, but that their further migration and relocation within the gonadal primordia was passive.

In 1934, Goodrich, Dee, Flynn and Mercer found that in Lebistes reticulatus the primordial germ cells could be recognized in the mesentoderm of the early gastrula and that later they became incorporated in the embryonic shield, where they were divided into two groups by the formation

of the nerve wedge. They concluded that in this form the primordial germ cells gave rise to the definitive sex cells and that none was derived from stromal cells.

Maschkowziff (1934) claimed that in the Salmonidae and Acipenseridae the primordial germ cells are of two sizes and are first located in the gut entoderm. He further claimed that the primordial germ cells are dimorphic from the very beginning and that this dimorphism is dependent on the amount of yolk present. He says (pp. 65-66):

> "Die primären Geschlechtszellen sind von zweierlei Typus:kleine und grosse; ihr Unterschied wird durch die verschiedene Quantität an Dotter im Protoplasma hervorgerufen. ... Bei grosser Dottermenge dominiert der weibliche Faktor, d.h. die grossen Ureier sind Geschlechtszellen vom weiblichen Typus. Bei einer kleinen Quantität Dotter dominiert der mannliche Faktor, d.h. die kleinen Ureier sind vom männlichen Typus."

He reported that the germ cells migrate from the gut entoderm through the dorsal mesentery to the region of the nephrostome, where the gonad later developed. I believe that this author's work is unique in maintaining that there is a dimorphism in the primordial germ cells.

Bennington (1936) found that in the adult male of the Siamese fighting fish, Betta splendens, the germ cells arise from residual cells which were left over from the previous spermatogenic cycle. These residual cells are lineal descendents of the primordial germ cells. Bennington's observation agrees with the conclusions reached by Hann (1927) for Cottus.

Odum (1936) found that in the toadfish (Opsanus tau) the primordial germ cells occur in two uniform strands in the posterior region of the coelom. Sex is related to the fate of the strand. In the male the posterior portion develops, and in the female the anterior portion develops. He found some degeneration of the primordial cells, and in the female a wholesale transition of stromal cells into germ cells.

Dildine (1936) found the germ cells in the mesentoderm of Lebistes

reticulatus soon after gastrulation. Most of them lay mesially, in the mesoderm of the prospective somite. A few, however, lay lateral to the somite and moved into the lateral plate as it was differentiated. He suggested that the mesial germ cells may have passed through the somite during their migration into the lateral plate. Coincident with the formation of the coelom, all of the germ cells moved into the splanchnic mesoderm and shifted dorsad around the gut and through the dorsal mesentery.

Moore (1937) traced the origin of the germ cells in Salmo irideus Gibbons, found that they were set aside early in the development of the embryo, and that they were first located in the splanchnopleure of the hindgut. The earliest stage in which they were recognized was in the nine-day embryo. From this place of earliest recognition, they migrated through the dorsal mesentery to the germ ridge, and after arriving there they migrated craniad. As a result of this migration craniad, the germ ridge came to consist of certain regions containing germ cells and other regions with no germ cells. He concluded that the primordial germ cells gave rise to both ova and spermatozoa. Regarding the origin of the primordial cells, he says: "... and since there is no evidence of differentiation of the germ cells from somatic cells, it is possible that these cells are of blastodermic origin" (p. 107).

From the foregoing accounts it is apparent that considerable diversity of opinion exists regarding the origin, migration, and ultimate fate of the germ cells in fishes. While it is not possible to discuss all of them in this paper, some will be given further consideration.

Observations on Micropterus salmoides salmoides

1. Characteristics of the Germ Cells

Although the descriptions of germ cells of vertebrates differ in detail, certain general characteristics have been found to obtain. In vertebrates the early germ cells usually are characterized by yolk granules and attraction spheres in their cytoplasm, by their large size and definite nuclear and cellular membranes, by their darkly staining nuclei and hyaline cytoplasm, and by their ability to migrate independently. Less commonly observed features of them are the extrusion of plasmosomal bodies into the cytoplasm (Dodds 1910), the difference in the shape of their mitochondria as compared to somatic cells (Rubaschkin 1910 and Tschaskin 1910), the possession of vitelline bodies (King 1908 and Okkelberg 1921), the peculiar configuration of their Golgi bodies (Woodger 1925), and a characteristic peripheral arrangement of the chromatin in the nucleus (Richards and Thompson 1921). Most investigators prefer to rely not on any particular characteristic for their identification but on the general appearance of the germ cell itself, because it has been shown that these several characteristics may vary with the species studied, with the type of fixation and stain used, and even with the physiological state of the germ cells at the time of fixation.

In the largemouth black bass the germ cells may be recognized by their large size (figs. 28, 31, 35 and 38), by their granular cytoplasm, by their definite nuclear and cellular outlines (figs. 27, 31, 35, 38 and 42), by their capacity for independent movement (figs. 31 and 32) by the presence of an attraction sphere during certain phases of their migration (figs. 29, 33, 34 and 38), and by the diffuse arrangement of the chromatin

in their nuclei as compared to that of somatic cells (figs. 33 and 39). No primordial germ cells were observed to possess yolk granules, a feature which agrees with the findings of Dodds (1910), who also failed to observe yolk granules in the primordial cells of Lophius. Dodds (1910, p. 579) remarks that "The germ cells of most vertebrates so far studied are up to quite a late stage filled with deeply staining yolk spherules which cause them to stand out prominently among the surrounding cells. In my preparations, they are marked in no such conspicuous manner."

It cannot be emphasized too strongly that in spite of the apparent distinguishing characteristics noted above, recognition of the germ cells comes only after many hours of observation during which they are traced through their various phases of migration in successive stages of development. As Blocker (1933, p. 118) has written, "It is necessary ... to take into consideration the changes undergone by the germ cells themselves, in order to follow them from their place of origin to their final location in the germ glands."

2. Size of the Germ Cells

In the largemouth black bass the germ cells vary in size in the various embryos and larvae. Their size was determined in a series of embryos, larvae and fry ranging from embryos thirty-two hours in age to fry thirteen millimeters in length. Measurements were made with a calibrated ocular micrometer under oil immersion at a magnification of 1350 diameters. These measurements were averaged for each embryo, larva and fry and the averages are presented in the following table. The number of measurements made in each developmental stage is given in parentheses.

Table 2

Table of Average Measurements of Germ Cells

in Microns, 1350 X

Stage of Development	Average Size in Microns
37-hr. embryo (9)	12.451 X 13.003
42-hr. embryo (12)	10.86 X 14.245
44-hr. embryo (15)	12.10 X 12.32
47-hr. embryo (10)	11.13 X 13.44
4.5-mm. larva (10)	11.09 X 13.45
11- and 13-mm. fry (8)	11.17 X 13.72

The size of the germ cells was least variable in the forty-four-hour embryo and was most variable in the eleven- and thirteen-millimeter fry. The germ cells were most nearly spherical in the forty-four-hour embryo and were least nearly spherical in the forty-two-hour embryo. The elliptical form encountered in the germ cells of the forty-two-hour embryo may be attributed to the fact that at that time they were migrating from the subintestinal yolksac extension into the splanchnic mesoderm below the gut (figs. 29 and 33).

Mention must be made of the size differences between the primordial germ cells and the cells with which they are most often confused, the hemocytoblasts (primitive blood cells). Figure 37 is a photomicrograph of a cross section through the yolksac extension of a forty-five-hour embryo from which its membranes were removed. During manipulation its ventral mesentery was slightly torn but its general relationships are still apparent. Here a germ cell and a cell identified as a hemocytoblast are seen lying side by side. The hemocytoblast is identical with those found in the primitive blood vessels. In an enlargement of the two cells (fig. 38), the attraction sphere of the germ cell is very clearly shown.

The granular cytoplasm of the primordial cell appears as a "cap" surrounding the nucleus. This cell is about 3.6 times as large as the hemocytoblast. By actual measurement the ratio of the volume of the nucleus to that of the cytosome is 9:4 for the hemocytoblast and 33:9 for the primordial germ cell. The nucleus of the hemocytoblast is relatively greater in volume as compared to the total volume of the cell than is that of the germ cell.

3. The Number of Primordial Germ Cells

A question that usually arises in an investigation of the germ cell history is that of the number of primordial germ cells. In the present study the germ cells were counted and their positions relative to the gut and the pronephric ducts were recorded for a series of embryos, larvae and fry ranging from the thirty-seven-hour stage to the thirteen-millimeter stage. Extreme care was exercised to obtain accurate counts. This was facilitated by the size of the germ cells themselves, which usually did not extend through more than two sections ten micra in thickness. The section containing the maximum portion of the nucleus arbitrarily was recorded as the one in which the germ cell was located.

The number of primordial germ cells was found to vary from individual to individual and from series to series of collections. As few as 21 were counted in a thirty-seven-hour embryo and as many as 105 in a thirteen-millimeter fry. The low number obtained in the thirty-seven-hour embryo undoubtedly should be attributed to difficulty of recognition, since as embryo and larval development progressed recognition of the germ cells became increasingly easier. It is logical to assume that the increase in number was due to division of the germ cells. However, a careful study

of numerous embryos, larvae and fry was made but in none of them was a germ cell seen to be dividing.

The usual relationship of the germ cells to the gut and pronephric ducts in a 3.4-millimeter larva is shown in table #3 (pf). The total number of germ cells recorded for this individual is 61, about the average number for larvae of this length. The number of sections, ten micra thick, cranial to the anus in which germ cells were found is noted in the left hand column. The number of germ cells in each section is recorded in the center column and their positions relative to the gut and pronephric ducts are recorded in the right hand column.

While counting the germ cells it was observed that in the 3.5-millimeter larvae they were concentrated near the midline and were restricted to an area 280 micra in length, beginning with the eighth section craniad of the anus and continuing craniad through the thirty-sixth section. In the 5.5- to 6.5-millimeter larvae the germ cells were distributed laterally, in the region ventral and ventrolateral to the pronephric ducts. In larvae of this length, the germ cells were restricted to an area 300 micra in length, beginning with the eighteenth section craniad of the anus and extending through the forty-eighth section. Text figure 10 is a graphic representation of the lateral and cranial shifting of the germ cells. In this figure, the midline of the larvae is designated by the solid center line of the graph. The graph is thus divided into right and left portions corresponding to the right and left sides of the larvae. The positions of the germ cells relative to the gut and pronephric ducts are plotted along the vertical axis and the number of sections anterior to the anus is represented on the horizontal axis. The solid circles represent germ cells of 3.5-millimeter larvae, and the open circles represent those of 5.5- to 6.5-millimeter larvae. In the 3.5-millimeter larvae

Text Plate 3

Explanation of Figure

10 A graphic representation of the distribution of the germ cells in 3.5-millimeter larvae and 5.5- to 6.5-millimeter larvae. The germ cells of the 3.5-millimeter larvae are represented with solid circles and those of 5.5- to 6.5-millimeter larvae with open circles. The positions of the germ cells relative to the gut and pronephric ducts is plotted along the vertical axis and the number of sections anterior of the anus on the horizontal axis.

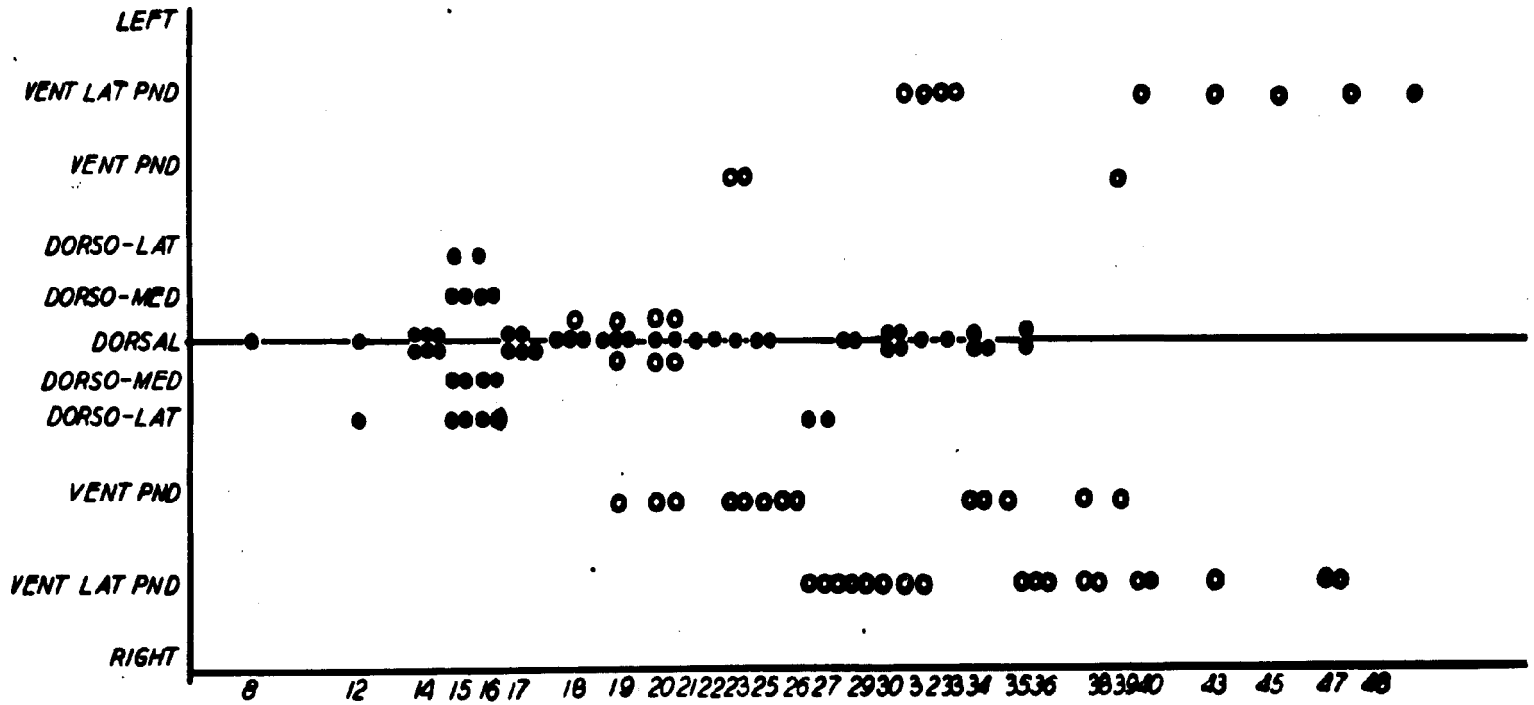
LEFT
VENT LAT PND
VENT PND
DORSO-LAT
DORSO-MED
DORSAL
DORSO-MED
DORSO-LAT
VENT PND
VENT LAT PND
RIGHT
8 12 14 15 16 17 18 19 20 21 22 23 25 26 27 29 30 32 33 34 35 36 38 39 40 43 45 47 48

Table 3

Germ Cell Count in a Prehatching Embryo

3.5 mm. in Length 46 to 47 Hours in Age

Section cranial of anus	Number of germ cells	Relative location
8	1	In yolksac extension ventral to gut.
9	0	
10	1	Ventrolateral to gut.
11	0	
12	0	
13	2	1 dorsomedial to gut, 1 dorsolateral to gut.
14	6	Dorsal to gut.
15	7	Dorsolateral and medial to gut.
16	7	Dorsolateral and medial to gut.
17	5	Dorsomedial to gut.
18	3	Dorsal to gut.
19	3	Dorsal to gut.
20	6	4 dorsal, 1 lateral, 1 dorsolateral to gut.
21	1	Dorsal to gut.
22	1	Dorsal to gut.
23	1	Dorsal to gut.
24	1	Dorsal to gut.
25	2	Dorsal to gut.
26	0	
27	1	Ventral to left pronephric duct.
28	1	Ventral to left pronephric duct.
29	2	Dorsomedial to gut.
30	0	
31	4	Dorsomedial to gut.
32	1	Dorsal to gut.
33	1	Dorsal to gut.
34	3	Dorsomedial to gut.
35	0	
36	1	Dorsomedial to gut.
Total	61	

the first germ cell was found in the eighth section anterior to the anus. With the exception of those in sections twelve, fifteen, sixteen, twenty-seven, and twenty-eight, the majority of the cells were situated near the midline or slightly lateral to it. In the 5.5- to 6.5-millimeter larvae the most posterior germ cell was found in the eighteenth section anterior to the anus and was situated near the midline. Farther craniad, however, the germ cells were observed to lie more and more lateral in position and were situated in the region of the definitive gonad, ventral or ventro-lateral to the pronephric ducts. From this graph it may be noted that the germinal area of the 3.5-millimeter larvae extends through 28 sections or 280 micra, whereas, that of the 5.5- to 6.5-millimeter larvae extends through 30 sections or 300 micra. The relative lengths of the germinal areas is about the same for the two larvae, except that in the 5.5- to 6.5-millimeter one it has shifted about 100 micra farther forward from the anus. This shift of the germinal area in the 5.5- to 6.5-millimeter larvae may be attributed in part to a migration craniad of the germ cells themselves and in part to a lengthening of that portion of the hindgut posterior to the germinal area.

4. History of the Germ Cells

A. General Consideration. The history of the germ cells in the largemouth black bass may be divided into two primary stages each of which may be subdivided into periods and phases (Table 4). These several divisions may be correlated, furthermore, with various life history stages (Table 4).

Table 4

The History of the Germ Cells
in the Largemouth Black Bass

Developmental Stage	Division of Germ Cell History
Blastoderm to 6.5 mm.	Pregonadal stage
Blastoderm to 32 hrs.	Premigration period including locus of earliest recognition
32 hrs. to 6.5 mm.	Migration period
32 hrs. to 47 hrs.	Yolksac extension phase
37 hrs. to 4.0 mm.	Splanchnopleure phase
3.5 mm. to 6.5 mm.	Retroperitoneal phase
6.0 mm. to adult	Gonadal stage
6.0 mm. to 3.0 cm.	Germ gland formation period
20 mm. to 3.0 cm.	The indifferent period
3.0 cm. to 4.5 cm.	Sex differentiation period

B. Premigration Period. In an embryo of sixteen to eighteen hours of development, about the time of blastopore closure, an excrescence of large cells was observed in the region of the dorsal lip of the blastopore (fig. 16). A careful study of sagittal serial sections revealed that these cells, or at least some of them, make their way into the embryo between the mesentoderm and the periblast (fig. 17). I believe that these cells are the forerunners of the primordial germ cells seen later

in the periblast below Kupffer's vesicle. This belief is supported by the fact that their size is about the same as that of the cells positively identified as germ cells. If this interpretation is correct, this excrescence represents the earliest stage at which I have been able to identify the germ cells in this species. Here they seem to be definitely segregated and set aside for a future migration into the embryo.

In an embryo of twenty-two hours of development cells similar to those in the dorsal excrescence were observed in the periblast in the vicinity of Kupffer's vesicle (fig. 27). These cells in the periblast measured approximately 12.0 by 12.5 micra. In later stages of development they come to lie in the caudal extension of the yolksac. It appears that as the tail differentiates and lengthens a subintestinal extension of the yolksac is carried back, with the result that the germ cells are shifted caudad. In embryos of thirty-two hours of development the germ cells are situated dorsally in the subintestinal yolksac extension (fig. 25). They possess a definite outline, granular cytoplasm, and measure approximately 12.5 by 13.0 micra. The migration of these cells from this extension of the yolksac is described in the following section.

C. Migration Period. The first phase of migration, the yolksac extension phase, was observed in embryos of thirty-seven hours of development. At this stage the germ cells were observed in apparent migration from the subintestinal yolksac extension into the mesoderm ventral to the gut. Figure 29, a transverse section through a thirty-seven-hour embryo at the level of junction of the yolksac with its caudal extension, shows two germ cells believed to be in migration. One is located in the dorsal portion of the yolksac extension and is passing into the mesoderm ventral to the gut. The other has completed its passage and is lodged in the

splanchnic mesoderm dextroventral to the gut. It is significant that the germ cells do not enter the gut entoderm, nor are they immediately associated with it. The impression is gained that the lowermost cell is apparently dissolving the mesodermal cell against which it is resting. It would appear that the germ cells possess some cytolytic property which enables them to pass through layers and masses of cells. However, they may merely wedge their way between the mesodermal cells. Each primordial germ cell possesses a centrosphere in the trailing side of the cytoplasm.

This first migration phase continues for several hours. A section of a forty-one-hour embryo, taken at the same level as figure 29, shows two germ cells in the mesentery ventral and to the left of the gut (fig. 26). The characteristic diffuse arrangement of the chromatin in the nucleus and the granular condition of the cytoplasm are evident. Convincing evidence of an amoeboid movement was observed in forty-four-hour embryos. In figure 30 a germ cell is seen projecting into the cavity of the yolksac extension ventral to the left pronephric duct. This cell is on its way from the extension into the mesentery above. A higher magnification of the pronephric duct region reveals that the germ cell possesses a blunt pseudopodial extension which projects into the mesoderm above (fig. 31). Figure 32, a still higher magnification of the region immediately adjacent to the cell, shows the extent of the pseudopodium.

In other forty-four-hour embryos the germ cells have migrated into the mesoderm ventral to the gut and are located to the right and the left of it. In figure 33, the difference between the nuclei of the germ cells and those of the somatic cells of the gut and surrounding tissue are clearly demonstrated. In a section somewhat posterior to that

represented in figure 33, a germ cell is seen in the dorsal part of the cavity of the yolksac extension just ventral to the right pronephric duct. The definiteness of its cellular outline and the presence of an attraction sphere to the right of the nucleus should be noted (fig. 34). Sections taken further caudad show that the germ cells lie in the mesentery ventral to the gut. In figure 35, representing a section almost at the posterior end of the yolksac extension, a germ cell occupies a position in the mesentery dextroventrolateral to the gut. Its outline is very definite and the cell stands out conspicuously among the flattened cells of the mesentery. It appears that the germ cells are capable of retaining their definite outlines even when surrounded by other tissue cells. This quality is retained throughout their history.

Text figure 8 represents diagrammatically the path of migration taken by the germ cells. The germ cells are indicated by black dots and are shown passing from the yolksac extension into the mesoderm ventral to the gut and then dorsad around the gut through the splanchnic mesoderm to the dorsal mesentery. This ends the first phase of migration.

The second phase of migration begins when the germ cells have entered the splanchnic mesoderm. In embryos of near hatching age, the germ cells were observed in the process of migration dorsad in the splanchnic mesoderm around the gut and are to be found in various positions in it. In embryos of this age the gut is a well-formed tube consisting of a single epithelial layer with an indiscrete basement membrane. A transverse section through the hindgut region of a forty-seven-hour laboratory hatched embryo shows two germ cells lying in the mesoderm (fig. 40). In other 3.5 millimeter larvae the germ cells are found in the splanchnic mesoderm ventral and ventrolateral to the gut. Their outlines are very distinct even though completely surrounded by mesenteric cells (fig. 42).

The section represented in figure 42 is taken posterior to that shown in figure 36 and is completely posterior to the yolksac extension. In this section four germ cells are present in the splanchnic mesoderm, two ventral to each pronephric duct. The basement membrane of the gut is very definite and it is clear that the germ cells are not located in the entoderm. Careful observation discloses that flattened mesodermal cells are present between the germ cells and the gut. The germ cells seem to be interposed between the layers of coelomic mesoderm. Thus, it appears that the germ cells are following the potential coelomic cavity as a route of migration.

In four millimeter larvae the germ cells lie in the mesentery dorsal to the gut. Larvae of this length are one to two days old and move about by vigorous lashings of the tail. Most of the changes which have occurred between the 3.4- and the 4.0-millimeter larvae are internal, involving the yolksac and the gut. The midgut is slightly curved to the left and the liver is beginning to form between the yolksac and the body wall. The coelom is beginning to form in the posterior trunk region.

Sections through the hindgut region of a 4.0-millimeter larva show that the germ cells are situated in the dorsal mesentery near the crest of the gut (fig. 44). Even at this early stage the germ cells are retroperitoneal and are ready to begin their third phase of migration. This relationship is better seen in 4.5- and 5.5-millimeter larvae. Sections of the hindgut of larvae of this length, two days after hatching, show that the germ cells now lie above the peritoneum. In figure 46, a section through the hindgut of a 5.0-millimeter larva, three germ cells lie ventral to the pronephric ducts, two on the right and one on the left. Considerable change has taken place in the gut and coelom as compared to the condition found in the 3.5-millimeter larvae. The coelomic spaces

are very well defined and the gut epithelium has assumed a columnar arrangement. The dorsal mesentery is relatively much thinner than before and there is some evidence of the formation of vascular channels.

The third migration phase, the retroperitoneal phase, was observed to begin in 5.5- to 6.5-millimeter larvae, three to five days after hatching. In the retroperitoneal phase the germ cells shift laterally from a concentration along the midline to a position ventral and ventrolateral to the pronephric ducts. The beginning of this shifting is observed in 5.5- to 6.5-millimeter larvae. In figure 47, a section of the hindgut of a 6.5-millimeter larva, four germ cells are represented. Two lie ventral to each of the pronephric ducts, slightly to the right and to the left of the dorsal mesenteric limbs. The epithelium of the gut has been thrown up into four major folds, two of which show in this section. In sections further craniad the germ cells are situated in a more lateral position and have reached the site of the future gonad (fig. 48). This migration phase is demonstrated graphically by text figure 10. In this figure the germ cells of 3.5- to 4.0-millimeter larvae are represented by solid circles and those of 5.5-to 6.5-millimeter larvae by open circles. From a midline concentration in the 3.5-millimeter larvae the germ cells have spread laterad and craniad in the 5.5- to 6.5-millimeter larvae so that they roughly approximate the definitive position of the gonads.

D. Germ Gland Formation. The condition of the germ cells in the 6.5- to 7.5-millimeter fry is much the same as that found in the 5.0-millimeter larvae. In the 8.0-millimeter fry, however, the germ gland begins to differentiate.

After reaching the germ gland site, the germ cells, now primary gonia, are invested with mesenchyme cells and are completely set off from

the surrounding tissue. Figure 51, a photomicrograph of the gonad of an 8.0-millimeter fry, serves to illustrate this condition. The gonium is surrounded by mesenchyme-epithelial cells derived from the peritoneum and subjacent mesenchyme. At first glance it appears to be naked on its coelomic side, but careful observation discloses it to be underlaid by thinly-stretched mesenchyme or peritoneal cells. The one-layered condition of the epithelium is rapidly changed to a two-layered one. This is brought about by proliferation of the cells already present in the epithelium and by further investment by mesenchyme cells (fig. 52). Cross sections of the gonad of an eleven-millimeter fry show that two layers of epithelial cells now invest the gonia (fig. 53). The inner layer may be interpreted as a follicular layer and the outer as a true epithelial layer. Later these two layers separate and form a cavity, the gonocoel, between them. In sections more anterior to that shown in figure 53, the gonad hangs suspended in the coelom by a delicate mesentery derived from the peritoneum, and in cross section has the appearance of a short-handled club (fig. 54).

In eleven- and thirteen-millimeter fry the gonad begins to grow through increase in size and number of the gonia. Fry of this length are ten to twelve days old, swim freely, and are quite bass-like. The gut possesses two or three coils and ends in the straight hindgut. The urinary bladder is beginning to form as an evagination from the joined mesonephric ducts and lies dorsal to the gut and dorsal mesentery, retroperitoneally. In this portion of the dorsal mesentery a genital sinus later forms, which is continuous with the gonocoel of the gonads. The swim bladder has grown caudad dorsal to the gonads, which now are suspended from its ventral surface as in the adult.

From time to time new gonia are added to those already present so

that cysts of gonia are formed (fig. 50). This increase in number may be attributed to division, but since no mitoses have been observed it is possible that the increase is due to a continued migration from behind. The gonad of the eleven- to thirteen-millimeter fry is not a continuous strand of germ cells but is made up of aggregations of germ cells and intervening regions devoid of them. In gross appearance the gonad resembles a chain of beads spaced at long intervals. Subsequently, there is a filling in of the free spaces, and by the twenty-millimeter stage the gonad has the gross appearance of a continuous strand. Up until this time the gonad has been more or less a solid mass. Gonocoel formation is instituted by the separation of the epithelial layers of the gonad, leaving the germ cells contained within the epithelial layer which lies on the mesenteric side (fig. 55). About the time that gonocoel formation begins, the blood supply to the gonad is established and circulation of blood is indicated by the presence of numerous blood cells in the gonadal vessel located in the mesentery. Fry-fingerlings twenty millimeters in length are approximately twenty-five to thirty days old, and have attained the major characteristics of the species.

5. Sex Differentiation

A. General Statement. The period of sex differentiation is that period during the development of an animal when sex first becomes recognizable. The sex chromosomes are generally conceded to be the primary determiners of sex. Apparently, however, they do not always exert their full influence in the early stages of development since numerous cases of juvenile hermaphroditism have been observed. Such conditions have been reported by Hargitt (1905) for several amphibians, by Grassi (1919)

for the eel, by Okkelberg (1921) for the brook lamprey, by Mrsic (1923) for the rainbow trout, by Risley (1933) for the musk turtle, and by Dildine (1936) for the guppy, and by others.

The determining factors seem first to exercise their influence on the gonads, since sex differences are first recognized in the structure of the gonads and the germ cells.

B. The Indifferent Gonad. Between the twenty-millimeter stage and the three centimeter stage the gonads, while potentially male or female, pass through an indifferent period. During this period there is a marked increase in their size, brought about by an increase in the stroma and by multiplication of the germ cells. The gonads, however, are predominantly stromal in composition. The gonocoel undergoes considerable enlargement and becomes more or less S-shaped, indicating differential growth in certain portions of its walls (fig. 56). In some regions the gonia are in division while in others they are in an interphase. An anterioposterior gradation of development is discernable during this period. Somewhat later, sex becomes microscopically recognizable. This condition will be discussed in the section devoted to the development of the ovary and the testis.

C. Development of the Ovary. Sex is first microscopically distinguishable in the gonads of fingerlings of 3.0 centimeters in length. In females of this size some of the gonia transform into oöcytes and undergo growth. Coincident with the growth of the oöcytes there is a marked increase in the amount of stroma in the gonad, resulting in the formation of a tunica ovarii several layers in thickness (fig. 57). The mesovarium is quite broad and contains near its base both an artery and a vein. The gonocoel, now properly termed an ovocoel, is irregular in outline and

is lined with squamous epithelium. A study of serial sections reveals that the ovocoels of the two ovaries fuse posteriorly to form a common sinus which continues into the dorsal mesentery of the hindgut as a median oviduct (figs. 58, 59, & 60). When traced forward, the ovocoels end blindly within the ovaries.

In the ovaries of the more mature fish of 3.5 centimeters in length, the oöcytes occur singly or in cysts in follicles formed by a single layer of stromal cells. The oöcytes are contained within folds of stroma which project into the ovocoel (fig. 61). Transverse and longitudinal sections of the ovary reveal that the lamellae have a definite pattern of arrangement and are most highly developed in the middle third of the ovary. The oöcytes in fish 3.5 centimeters long are two to three times as large as those in fish 3.0 centimeters long and show the beginnings of primary yolk formation (fig. 63). In certain oöcytes in more advanced stages of growth a light area of cytoplasm occurs directly around the nucleus surrounded by a darker peripheral area where yolk formation has begun (fig. 64).

In the 4.5-centimeter stage the sexes are distinguishable in gross dissection. Figure 71, a photograph of the gonads of a 4.5 centimeter male and female, shows that the ovaries appear as enlarged distended structures along the ventral surface of the swimbladder, whereas, the testes appear as similarly situated thin strands. Sections of the ovary at this stage reveal that certain oöcytes appear to be growing more rapidly than others. In those oöcytes undergoing rapid growth, the nucleus assumes a condition similar to that found in ripe ova. The chromatin loses its reticular structure and is broken up into numerous rounded bodies arranged peripherally along the nuclear membrane (fig. 62). This arrangement is probably significant in connection with yolk formation.

In addition to oocytes in advanced stages of growth, numerous smaller oocytes and even oogonia are present (fig. 62).

In fingerlings 6.0 to 8.0 centimeters in length the ovaries appear as distended elongate bodies located posteriorly in the coelom (fig. 72). The thin-walled sinus is even more evident than in previous stages (fig. 72). Sections of ovaries at this time reveal secondary yolk formation in certain of the larger oocytes. This is indicated by the formation of vacuoles in the darker peripheral cytoplasm. The later history of the egg has not been followed in this investigation.

D. Development of the Testis. The organization of the testis apparently proceeds at a slower rate than does that of the ovary. Not until the 4.0 centimeter stage is reached does its differentiation become recognizable. The testis of fingerlings of this length bears little resemblance to that of older fish. The early testis differs structurally from the early ovary in the size of the gonocoel, the size of the gonia (spermatogonia), and in the relative amount of stroma present. The gonocoel, now properly termed the testocoel, is much smaller than that found in the early ovary and is lined with a low, irregular cuboidal epithelium instead of a squamous epithelium as is the ovocoel. The testis is predominantly germinal in composition. It lacks the thickened tunic which covers the ovary and is suspended in the coelom by a delicate mesorchium (fig. 65). No germ cells lie in the epithelium, but lie in the subjacent stroma and deeper in the gonad. Some of the germ cells occur in clusters and others occur singly, but all are surrounded by stromal cells. Most of the spermatogonia are similar in size but occasionally one is found that is slightly larger than the others. Whether these occasional ones are enlarged spermatogonia or are aberrant oocytes has not been

determined. Some of the germ cells appear to be radially arranged around a potential lumen. Even so, each is surrounded by stromal cells. The testis of this and later stages of development (fig. 73) exhibit an anterioposterior gradation in development, being more highly developed posteriorly. A clue to tubule formation, which occurs in this species, is obtained from sections taken posterior to that shown in figure 65. The testocoel gives off numerous branches which penetrate the stroma between the germ cells so that they come to lie around them (fig. 66). Since these branches project in all directions and take tortuous paths, cross sections of them are obtained in any plane. This explains the radial disposition of the germ cells described earlier. Morphologically the testocoel would correspond to a primary collecting duct and its branches to secondary and tertiary collecting ducts.

In the 5.0- to 6.5-centimeter stage the testis has reached a definite tubular form and is enlarged and dorsoventrally flattened (fig. 67). The germ cells are located in the walls of the tubules, occur singly, and are separated by stromal cells (figs. 69 and 70). The tubules consist, then, of both spermatogonia and supporting stromal cells. The germ cells appear to lie in but not to form the epithelium of the tubules (fig. 70). The epithelium of the tubule is derived from the testocoel, and each tubule is delimited by a connective tissue layer of stromal cells.

In this as in the preceeding stage, most of the spermatogonia are of approximately the same size. The testis at this stage also shows an anterioposterior gradation in development and is more highly organized, posteriorly. Sections taken through the posterior portion of the testis show more definite tubule formation (fig. 68). The primary collecting

duct lies near the mesial side of the testis (fig. 68) and fuses posteriorly with a similar one from the other testis. The common duct thus formed continues as a vas deferens to its external aperture near the opening of the ureter.

For an account of further changes which occur in the ovary and testis prior to and during a seasonal cycle, reference is made to a recent paper by James (1946).

Discussion

Segregation of the Germ Cells

It is generally agreed that the germ cells retain certain generalized characteristics or properties which serve to distinguish them from somatic cells. The nature of these characteristics and of the germ cells in general will now be considered.

Eigenmann (1891), from work on Micrometrus, believed the germ cells to be primitive cells because he could trace them back into early stages of development, probably to the fifth or sixth segmentation. The works of Wheeler (1899), Beard (1900-1902), Woods (1902), Fedorow (1907), Allen (1911), Dodds (1910), Bachmann (1914), Okkelberg (1921), Richards and Thompson (1921), Hann (1927), Goodrich et al (1934), Maschkowziff (1934), Bennington (1936), Dildine (1936), Moore (1937), and others all point to an early segregation and to a more or less primitive or embryonic condition of the primordial germ cells. Some investigators have found no morphological basis on which to pass a judgment and contend that the difference between somatic and primordial germ cells is merely a physiological one. Others have been influenced by certain physical characteristics and believe that the primordial germ cells are undifferentiated. Among these characteristics are their yolk spherules, their large size, their static condition for long periods of time, and their capacity for independent movement. Still others believe that the primordial germ cells are undifferentiated because they possess an attraction sphere or because of their locus in the embryo.

Dodds (1910) found that there was an extrusion of plasmosomal material from the nucleus in the early germ cells and for this reason

concluded that they were less primitive than the somatic cells in which such extrusions were not noted. Jordan (1917), in describing the cells of the germinal area in the loggerhead turtle, said that they represented a low grade of differentiation from cells of the blastoderm, inferring that they are undifferentiated cells. Because the germ cells remain unchanged for a long period of time whereas the somatic cells become smaller, lose their yolk and are organized into tissues, Okkelberg (1921) thought that the germ cells retain certain embryonic characteristics which serve to distinguish them from somatic cells.

The primordial germ cells of Micropterus salmoides salmoides are recognized early in development, and, on the same basis that others have judged the primordial cells in various forms to be embryonic they also may be classed as undifferentiated cells. They are large in size and possess a distinct attraction sphere during certain phases of their history, are capable of independent movement, and are able to retain their cellular outlines when in contact with other cells, a property often lost by somatic cells. They do not possess yolk spherules but this lack is accounted for by the fact that few blastoderm cells in the bass do contain yolk. Most forms whose primordial cells contain yolk develop from eggs with holoblastic or deep meroblastic cleavage. In the bass the blastodisc alone cleaves and little or no yolk is included in the blastomeres.

The appearance of the excrescence at the dorsal lip of the blastopore, late in gastrulation, marks the time when the germ cells are first recognized. It has not been possible to distinguish any potential germ cells earlier than this. However, this does not preclude the possibility that they may have been segregated earlier. Although the manner of

formation of the excresence was not determined, it appears to result from an unequal rate in the streaming movement of the cells during gastrulation causing a piling up of the cells at the entrance to the blastopore. The position of the cells in the blastoderm with respect to the streaming movement might determine which are to become germ cells, though the accident of position could hardly be regarded as a determining factor. Not all of the cells of the excresence, but only those which succeed in making their way into the periblast beneath the dorsal lip, become germ cells. Those remaining outside may degenerate or become incorporated in the caudal mass. One reason for thinking that they might become included in the caudal mass is the fact that they remain surrounded by the epidermal stratum. It is only after a certain degree of differentiation in other cells has occurred that the primitive condition of the germ cells makes them conspicuous. Actually it matters little whether we can trace the germ cells back to the undifferentiated blastoderm or beyond, so long as we can show that they never have assumed any somatic duties.

Body and Germ Cell Relationships

It has been shown that late in the embryonic development of vertebrates the germ cells come to lie in the coelomic epithelium covering the gonad, the germinal epithelium. This fact led to the concept that the primordial cells had developed from epithelial cells or were transformed stromal cells. Several students of this problem in fishes have found what they consider to be a transformation of stromal and epithelial cells into germ cells (Böhi 1904, Essenberg 1923, Van Oordt 1924, Foley 1927, Wolf 1931, and Odum 1936). This concept has been applied to many other

vertebrates than fishes, as table 1 indicates. The evidence presented by many investigators in support of this concept is almost convincing. Nevertheless, the following statement made by Beard (1902 C, p. 691) on this subject, is still applicable: "The change from epithelial cell to germ-cell, though asserted times without number, has never really been depicted, and in all probability it has never actually been observed. Indeed, it does not exist." A few investigators admit that either the fate of the primordial cells is doubtful, or that they give rise to one or the other of the sexual elements. It seems quite improbable, if not impossible, for definitive sex cells to be derived from primordial elements in one sex and from somatic elements in the other.

A germinal epithelium, in the sense that it has been described by various authors for certain reptiles, birds and mammals, is nonexistent in the bass. For this animal the term "germinal epithelium" has no significance other than to designate that particular portion of the coelomic epithelium which overlies the gonads. It has already been shown that the primordial cells, after reaching the primordium of the gonad, remain as discrete elements and form no part of the epithelium. From the very beginning they lie above the epithelial cells of the coelom. No evidence of a transformation of stromal or epithelial cells into germ cells was observed to occur in either the testis or the ovary of the bass. Neither is there any indication of a degeneration of the primordial cells in either sex, as has been reported to occur in one or both sexes of some other fishes (Essenberg 1923, Van Oordt 1924, Wolf 1931, and Odum 1936). Therefore, I believe that the definitive sexual elements of both sexes arise only from the lineal descendents of primordial germ cells.

Migration of the Primordial Germ Cells

Two of the most interesting features of the germ cell history are the path and mode of migration of the primordial germ cells. The path usually described for higher vertebrates leads from the gut entoderm into the splanchnic mesoderm, thence dorsad through the dorsal mesentery and laterad to the germ gland primordia. This same general path of migration has been observed to hold for some fishes, particularly those which develop from eggs that undergo holoblastic cleavage such as Amia, Lepisosteus, and Acipenser (Allen 1911 and Maschkowziff 1934). Notable variations among the fishes also have been reported. It may be mentioned that in Lophius (Dodds 1910) and Lebistes (Dildine 1936) the germ cells were said to pass through the myotomes. Most investigators, however, have found that in fishes the path of migration of the primordial germ cells leads through one or both layers of the lateral plate mesoderm and not through the gut entoderm. One investigator, Wolf (1931), found that the primordial cells of Platypoecilus maculatus migrate through the somatic layer of mesoderm and pass mesiad into the gonad primordia.

The path taken by the primordial cells of Micropterus deviates not only from that usually described for the higher vertebrates but also from that usually reported for the fishes. The early location of these cells in the caudal extension of the yolksac and their subsequent migration through and between the layers of coelomic mesoderm are unusual. A similar but probably not a homologous condition is found in the human embryo of thirteen somites (Witschi 1948). Witschi found that the primordial germ cells of the human could first be identified in the entoderm of the caudal allantoic extension of the yolksac. The similarity of this extension with the caudal extension of the yolksac in the bass is worthy

of note (see fig. 1, Witschi 1948), though it seems improbable that these two yolksac extensions are homologues. If we consider, however, that in the largemouth black bass the periblast has taken over the function of the yolksac entoderm, the homology of these parts then is not impossible. The primordial cells of both the bass and the human actively migrate from the yolksac entoderm or the yolksac extension. Those of the human pass into the splanchnopleure and gut entoderm, and those of the bass pass into and between the layers of coelomic mesoderm. If Witschi's statement that "The gut endoderm is rather an obstacle than a center toward which free germ cells might direct their movements" (p. 77) is correct, then the condition found in the bass, wherein no germ cells become located in the gut entoderm, would represent a more basic vertebrate pattern than that found in the human and other higher forms. According to Witschi those germ cells of the human, and possibly other higher vertebrates, which occur in the gut epithelium get there quite accidentally, and as a result their migration is either retarded or stopped.

Three major theories have been advanced to account for the migration of primordial germ cells. The first of these is that cells migrate actively by amoeboid movement; the second is that they are passively carried along by shifting tissue masses, and the third is that they are carried by the blood stream.

According to those who uphold the first theory, the primordial cells are capable of moving between other cells and through membranes. Beard (1900-1902), Allen (1911), Bachmann (1914), Reinhard (1924), Wolf (1931, and Moore (1937), and others have described an active amoeboid migration of germ cells in fishes. This type of migration has also been described for other vertebrate forms by Allen (1906), Jordan (1917), Woodger (1925),

Dantschakoff (1931 a), Cheng (1932), Risley (1933), Blocker (1933), Everett (1943), Witschi (1948), and others.

Those who support the second type of migration discount the possibility of an amoeboid migration, contending that it is improbable that the germ cells possess the ability to migrate through tissue and to traverse distances of several millimeters in order to reach the germ gland primordia. Simkins (1923, p. 268), who supports the germinal epithelium theory, does not believe that the germ cells can "set out on a journey over several millimeters of intervening tissue, cross barriers and penetrate membranes, become carried away in the blood stream, perishing against obstacles they cannot surmount, until the survivors are at last safe in the fundament of the genital gland." With regard to the distance of migration, the only instance in which this might be even as much as one millimeter is in birds. It should be noted that they have a special type of migration. Even in the human embryo the total distance traversed by the germ cells is not more than 0.5 of a millimeter (Witschi 1948). In mouse embryos, Everett (1943) found this distance to be not one or several millimeters but only a small fraction of a millimeter.

Some investigators think of the migration as being partly active and partly passive; or, as resulting from the normal processes of growth Dodds (1910), Okkelberg (1921), Richards and Thompson (1921), Hann (1927), and others. Richards and Thompson (1921) believe that the term "migration" is not appropriate and suggest the term "translocation" as a substitute.

The third theory, or vascular migration, has been described only in birds. Several investigators who have studied the problem of germ cell migration in these forms have either observed morphologically or demonstrated experimentally that the primordial cells actually enter or

become entrapped in the vascular channels of the splanchnic mesoderm of the area vasculosa and are passively carried to the germ gland region (Swift 1914, Reagan 1916, Richards, Hulpieu and Goldsmith 1926, Goldsmith 1928, Dantschakoff et al 1931, Blocker 1933, Dantschakoff 1935, and others). All have observed that after circulating in the blood stream for some time the germ cells become lodged in the capillaries near the gonad primordia. Several theories have been advanced to account for this. The oldest of these is the chemotatic theory. According to its proponents (Swift 1914, Firket 1914, and Reagan 1916), a chemotatic substance is elaborated by the gonadal primordia and attracts the germ cells. More recent workers, however, believe that aggregation of the germ cells is the result of mechanical blockage of the small capillaries by the large turgid germ cells (Dantschakoff 1931a and b, 1935, and Blocker 1933). Although they differ on the cause, all of these investigators agree that after the germ cells become lodged in the capillaries of the gonad region they assume active amoeboid movements, and by this means leave the blood vessels and migrate through the mesentery and adjacent areas to the gonad primordia. More recently Dantschakoff (1935) treated chick embryos with sublethal doses of X-ray and produced large phagocytic cells that were approximately the same diameter as primordial germ cells. She found that these cells acted in the same way as the primordial germ cells and ultimately came to rest in the gonads. She is of the opinion that the mechanical factors of size and consistency, together with reduced blood pressure, are primarily responsible for the retention of the germ cells in the capillaries of the gonad area.

As might be expected, not all of the cells identified as primordial germ cells reach the germ gland primordia. In some forms the germ cells

have been observed to take rather aberrant courses in their migrations. Some get "lost" in the mesenchyme of the dorsal mesentery and in the splanchnopleure and undergo either somatic differentiation (Rabl 1896, Von Berenberg-Gossler 1914) or degeneration (Beard 1902c, Jordan 1917). Others become cast off into the coelomic cavity (Beard 1902c) or into the lumen of the gut (Okkelberg 1921) and undergo degeneration. Still others have been observed to form cysts in various parts of the body. Some investigators believe that aberrant accumulations of these cells develop into dermoid cysts called embryomas. Beard (1902c) seems to believe that "an embryoma, at whatever period it appear, is an instance of the development of an additional primary germ cell, which in all its hereditary character is the exact counterpart of that primary cell, by whose unfolding the individual harbouring the 'dermoid' arose" (p. 672). Okkelberg (1921) also found aberrant cysts of primordial germ cells in the fat body of the brook lamprey. However, he does not say what becomes of them, other than that they probably degenerate. Other investigators have recorded the presence of aberrant germ cells but attach no particular importance to them.

My observations on the largemouth black bass support the theory of amoeboid migration of the primordial germ cells. Their movement is characterized by the formation of pseudopodial extensions which are usually blunt. This has also been demonstrated by Woodger (1925), Dantschakoff (1931a), and more recently by Witschi (1948). Situated as they are in early stages of development, there is little chance for the primordial germ cells to be shifted around by so called normal processes of growth. Their easiest course is to migrate independently, and this I believe they do. The likelihood of their being incorporated within any

blood vessel during its formation seems very slight. The form of the cells suggests that they are most active during the early phases of migration. After penetrating the splanchnic mesoderm their activity apparently is reduced and they assume a more or less rounded shape. The fact that the cells are able to assume a rounded form after an irregular one points towards independent movement. Some investigators seem to think that because the germ cells were seen by them as large rounded cells in the splanchnopleure they were incapable of independent movement. But as Allen (1906) has pointed out, the migration of the germ cells in the splanchnopleure might be so slight that irregularities in their shape would be imperceptable even to the most trained observer. It seems inconceivable that the germ cells in the bass should migrate in any other fashion than by independent movement. There is undoubtedly much wasted effort by these cells and Witschi (1948) probably is correct when he writes that "Much of their migration represents compensating movements against the stream of growth that tends to carry them away from their goal" (p. 77).

At no time during their history are the germ cells very far distant from the germ gland primordia, and the distance they traverse in relation to the amount of time they take for it is very minute. No lost or wandering germ cells were observed in the bass. The path of migration of the germ cells apparently is determinate. From the very beginning of their migration they seem to be under the control of some directing force. We can only speculate as to whether this force is chemotropic or mechanical. The supposition that it is mechanical is strengthened by the fact that the primordial cells make use of the potential coelomic space, and thus would be guided dorsad. This still does not explain why the cells enter the splanchnic mesoderm rather than the somatic mesoderm. If they

entered the latter they would be retroperitoneal from the very beginning and the total distance to be traversed would be considerably less. Witschi (1948) favors the idea of a chemotropism as the directing force in the migration of the primordial germ cells in the human. This concept, as he pointed out, is strengthened by the fact that leucocytes possess very definite chemotropic tendencies (Chambers and Grand 1936) and their activities have been likened to those of primordial germ cells (Dantschakoff 1936).

No attempt was made in this study to ascertain the number of primordial germ cells reaching the gonad primordia. Allen (1911) reported that only about half of the cells of Lepisosteus reach the gonads, whereas for Amia he concluded almost all are successful. He was unable to account for the difference in the two forms. Others who have made statistical studies have found that the number varies to such an extent that no generalized statement can be made.

The Periblast and the Primordial Germ Cells

During a short period of their history the primordial germ cells may be found in the periblast of the caudal extension of the yolksac. The periblast serves as a storage place for the cells from the time they become lodged there, early in development, until they begin their migration. Because of the type of development which occurs in the bass and certain other bony fishes, the periblast together with the yolk may be considered as being extra-embryonic. It is extra-embryonic, not in the sense that it lies outside of the embryo, but in the sense that it takes no material part in the formation of the embryo. Since this is the case, the germ cells, situated as they are in the periblast, may be considered

as being extra-embryonic also and are thus totally removed from any early chance inclusion in the soma.

Richards and Thompson (1921) found a close association between the periblast and the primordial germ cells in Fundulus. They found numerous instances where the germ cells "lay half-buried in the periblast." However, they were unable to attach any significance to this relationship. On the other hand, Reinhard (1924) described the primordial cells of Scardinius as originating from giant cells found in the periblast. Although there is reason to believe that he may have misinterpreted his material, he may have been more nearly correct than some believe. If in error, his mistake probably was due to the fact that he did not trace the giant cells back far enough and therefore made a natural but not too logical assumption that they originated from periblast nuclei. Hann (1927) observed these giant cells in Cottus and remarked that "Although the periblast nuclei are in close proximity to the giant cells, there is no evidence that the giant cells are derived from them" (p. 441). Hann believed the giant cells to be of entodermal origin. Wolf (1931) first located the germ cells in Platypoecilus maculatus between the periblast and the ectoderm but doubted whether they had come from the periblast. Most investigators have managed to avoid the issue and make no statement regarding this relationship.

In my opinion the relationship that exists between the periblast and the primordial germ cells in Micropterus salmoides salmoides is comparable to that which exists between the primordial cells and the peripheral yolksac entoderm of other vertebrate forms. This concept has as its basis the assumption that the periblast in Micropterus has replaced the yolksac entoderm of higher forms, in both structure and in function.

Multiplication of the Primordial Germ Cells

In this study the question arose as to whether the primordial cells divide prior to reaching the germ gland primordia. If they do not, how does one account for their apparent increase from one stage to the next? Most investigators of germ cells in fishes find that they do not divide during migration (Eigenmann 1891, Beard 1900-1902, Woods 1902, Böhi 1904, Allen 1911, Dodds 1910, Okkelberg 1921, Richards and Thompson 1921, Hann 1927, Dildine 1936, and Moore 1937). On the contrary, numerous workers have reported that there is no apparent cessation of mitoses during the migration period, and many have described primordial germ cells in mitosis. It is generally agreed that cells do enlarge prior to division and that their cytoplasm has a tendency to take a lighter stain. Since both of these characteristics, large size and light-staining cytoplasm, have been used as criteria for the identification of primordial germ cells, there are some (Simkins 1923 and Hargitt 1925) who would have us believe that the large rounded cells identified as primordial germ cells are nothing but somatic cells preparing to divide. With this thought in mind, I examined my material very carefully. Certain large cells were observed. When followed through a complete division, however, the resulting cells were found to be somatic cells and not primordial germ cells. Measurements were made of these large cells, and it was found that even when most enlarged, they did not compare in size with primordial germ cells. Therefore, I believe that I am justified in concluding that these enlarged cells were not primordial germ cells in mitosis.

Since the primordial germ cells of the largemouth black bass do not divide during their migration, the variation in their number is an individual characteristic. The fact that apparently they do increase in

number from one stage to the next, I believe, may be attributed to a sequence of events such as Dodds (1910) observed in Lophius wherein the germ cells became more conspicuous as differentiation of the soma increased. A similar condition was observed by Moore (1937) in the rainbow trout. His own statement (p. 107) concerning it is:

> "In the 11-day embryo there are many more germ cells than in previous ages. Since no mitotic germ cells occur and since there is no evidence of differentiation of germ cells from somatic cells, it is possible that these cells are of blastodermic origin just taking on the distinctive characteristics of primordial germ cells."

Number, Size and Distribution of the Primordial Germ Cells

According to Beard's (1902b) hypothesis, one would expect to find a relatively constant number of primordial germ cells in any individual of a particular species. This number, according to him, could be expressed by the formula 2^n-1. For Petromyzon, Beard (1902b) found the number of primordial germ cells to be thirty-two minus one. Most investigators have found that their findings regarding the number of primordial germ cells could not be reconciled with Beard's formula. Eigenmann (1896), not yet aware of Beard's hypothesis, found that in Cymatogaster there was a striking variation in the number of primordial cells in different larvae and attributed these differences to individual variation. Allen (1907), in his statistical study of the sex cells of Chrysemys marginata, reached similar conclusions and says: "His Beard's conception of a specific number of sex cells expressed by the formula 2^n-1 during these early stages certainly is not borne out by the facts observed in Chrysemys, in which the number of sex cells ranges all the

way from 302-1744" (p. 395). In a later paper Allen (1911) found that the number of primordial germ cells of Amia and Lepisosteus varied considerably and followed no specific formula.

Dodds (1910) also found that in Lophius the number of primordial cells varied from one individual to the next and definitely did not follow Beard's "Numerical Law of Germ Cells." Bachmann (1914) found that in Ameiurus nebulosus the number of primordial cells ranged from twelve to thirty-four, with an average of about twenty-three. For Fundulus heteroclitus Richards and Thompson (1921) found sixty-seven to be the average number of primordial cells. In Cottus bairdii (Girard) this variation was reported to range from one to eighty (Hann 1927). In Platypoecilus maculatus an even more extreme variation of from forty to five hundred and forty-three was reported (Wolf 1931). Although Goodrich et al (1934) found the number of primordial germ cells in Lebistes to range from thirty-seven to sixty-six, Dildine (1936) for the same species found the range to be from seventeen to thirty-five. For the rainbow trout, Salmo irideus (Gibbons), Moore (1937) reported the number of primordial germ cells to be eighty-four.

As was stated in a preceeding section, the number of primordial cells in Micropterus salmoides salmoides varied from individual to individual. This variation (20 to 105) is so extreme that in my opinion there is little chance that Beard's law would find any application here. I find that there is less variation among individuals of the same egg masses than among those of different egg masses. Apparently this is a common occurrence and most likely is due to congenital factors (Hann 1927 and others). No overall average number of primordial germ cells was determined for Micropterus; however, I believe that if a careful statistical

study were made, it would be about sixty.

Apparently the primordial germ cells of vertebrates vary less in size than they do in number. All those so far described would be classified as large cells. For example, Eigenmann (1891) found that the primordial cells of Micrometrus measured 13 to 18 microns in diameter. The dimensions of those of Lepisosteus ranged from 10.27 to 14.95 microns, and those of Amia ranged from 11.59 to 21.88 microns (Allen 1911). The diameters of those in the chick range from 14 to 22 microns, with an average of about 16 (Swift 1914). Bachmann (1914) reports that in Ameiurus the primordial germ cells vary in diameter from 14 to 18 microns. In the loggerhead turtle, Caretta caretta, their diameters were found to range from 13 to 20 microns (Jordan 1917). For Xiphophorus helleri their diameter is given at 11.8 microns (Essenberg 1923), and for Platypoecilus maculatus Wolf (1931) sets their dimensions at 11.5 by 8.5 microns. Two sets of measurements have been given for the primordial germ cells of Lebistes. Goodrich et al (1934) place their dimensions at 14 by 10 microns, and Dildine (1936) places them at 13 to 19 microns. The primordial germ cells of Rana cantabrigensis Baird range from 16 to 24 microns in diameter (Cheng 1932) and those of Sternotherus odoratus Lat. average 17 microns in diameter (Risley 1933).

The size of the primordial germ cells of Micropterus salmoides salmoides agrees quite well with those recorded for other vertebrates (see table 2). The variations in their size could be accounted for by change in shape during migration. One wonders why the primordial germ cells are so large. Their size might be explained on the basis of the need of reserve food material, yolk etc., an explanation which might suffice for lower animals and certain invertebrates. But in higher forms,

especially those whose primordial cells contain very little or no yolk, their magnitude may be attributed to a retention of the blastomeric qualities they possessed when first segregated. Granting this, by comparing their size with that of early blastomeres one might be able to determine the stage when they are first set aside in the blastoderm. This, I believe, might possibly be done for the largemouth black bass. The theory of such a procedure is based on the premise that the primordial germ cells remain morphologically static for a certain period after being set aside. This theory is not without foundation, since size is one of the least variable characteristics of any particular cell type. Eigenmann (1891) also expresses such a view in the following statement: "On comparing this size that of a primordial germ cell with segmenting eggs it is found that it agrees in size with some of the cells of an egg undergoing the ninth segmentation and in all probability it is a cell remaining unchanged from that stage" (pp. 484-485).

Maschkowziff (1934) finds that in both Salmo trutta and Acipenser stellatus the primordial germ cells are dimorphic. This condition is, according to him, brought about through differences in the amount of yolk contained by them. He believes that this difference in yolk content is indicative of the sex potentiality of the cells, the larger ones being female and the smaller being male. His views are unique in the literature and cannot be reconciled with those of any other investigators and this topic warrants further investigation.

Several investigators have found that the primordial germ cells are distributed unequally to the gonad primordia. Others have found an almost equal distribution and still others have found no set pattern. Eigenmann (1891) found that in Micrometrus no uniform distribution occurs. In some individuals there were more on the right side and in others there

were more on the left. In Lepisosteus there were usually more germ cells on the left side, whereas in Amia there were more on the right (Allen 1911). In the chick the germ cells show a decided preference for the left side (Swift 1915). Jordan (1917) found that in the loggerhead turtle the primordial germ cells were about equally distributed between the two sides. In Cottus bairdii G., Hann (1927) found an unequal distribution of the primordial cells, usually favoring the left. Cheng (1932) found that in Rana cantabrigensis there is no marked variation in the distribution of the cells. The left side is also favored by the primordial germ cells in Sternotherus odoratus (Lat.) (Risley 1933). Blocker (1933) found that the left side is favored by the germ cells in Passer domesticus L., as evidenced by gonad formation. Witschi (1935), for the same form, found 3 to 10 times as many germ cells migrating to the left primordium as to the right. An unequal distribution is reported to occur in Lebistes, as evidenced by primary gonia formation (Dildine 1936). Stanley and Witschi (1940) also found an asymmetrical distribution of the primordial germ cells in the sex gland primordia of the hawk.

The germ cells of Micropterus salmoides salmoides are distributed asymmetrically to the gonad primordia with the greatest number going to the right. In some 6.5- to 7.5-millimeter larvae, twice to three times as many germ cells are found in the right primordium as in the left. This asymmetrical distribution I believe may be attributed to a reduction in the potential migration area. Late in embryonic development in the region of the hindgut the caudal vein courses sinistroventrad around the gut in the splanchnic mesoderm and continues as the subintestinal vein, reducing the potential if not the actual migration area. The probability that this affects the migration of the primordial germ cells is revealed in their asymmetrical distribution.

Sex Differentiation

Numerous works dealing with sex determination and differentiation have appeared in literature. While a majority have supported the sex chromosome theory, they have reported in spite of this a condition of juvenile hermaphroditism. Such a condition seems to be the normal occurrence in certain cyclostomes, elasmobranchs and higher bony fishes. Since the literature on the subject is voluminous, only that dealing with cyclostomes and fishes will be considered, and that briefly.

One of the first works was that of Müller in 1875 on Petromyzon planeri. Some years later Cunningham (1887) found a condition of an ovotestis in Myxine glutinosa. He believed that the females were hermaphroditic, since he found very few males. Later investigators working on this and other related species found that the ovotestis was merely a condition of juvenile rather than functional hermaphroditism (Beard 1893, Price 1896, Dean 1897, and others). Juvenile hermaphroditism was first described in lamprey larvae (Petromyzon planeri) by Lubosch in 1903. Okkelberg (1921) found a similar situation in Entosphenus wilderi (Gage). Some recent studies dealing with sex differentiation and juvenile hermaphroditism in higher fishes are those of Essenberg (1923), Mrsic (1930), Goodrich et al (1934), Dildine (1936), and others. Essenberg (1923) has found what amounts to sex inversion along with juvenile hermaphroditism.

The mechanism of sex determination undoubtedly affects the time of sex differentiation. One would assume that if this mechanism were chromosomal, differences in the zygote would be established at fertilization. Apparently this is not the case. Sex differences do not always appear early even in those fishes wherein sex is known to be controlled by sex chromosomes. For example, in Lebistes reticulatus definitive

sex is controlled by an XX-XY mechanism (Winge 1922). Yet embryologically gonadal development is ovarian in all embryos (Dildine 1936). In the swordtail twice as many embryos show a tendency toward ovarian as toward testicular development, but about half of those with ovarian development later undergo gonadal regression and ultimately produce testes (Essenberg 1923). In certain other fishes sex seemingly is determined through the numerical superiority of one type of gonium, spermatogonium or oogonium over the other, with the result that the individual develops either ovaries or testes. In one instance sex apparently is determined by the action taken by the germ gland itself. If the anterior portion develops, a female results; if the posterior portion develops, a male results (Odum 1936, Opsanus tau).

Although in Micropterus salmoides salmoides there is a long period of gonadal development prior to sex differentiation, it is a period of indifference and not of juvenile hermaphroditism. The gonads never exhibit bisexual tendencies, and they never start development toward one sex, only to regress and develop toward the other. The gonads, I believe, are different from their formation and most likely this difference is due to a difference in the chromosomal constituency of the germ cells and even of the somatic cells comprising them.

Preliminary studies on sex ratios for this form support the theory of a chromosomal type of sex determination. However, this is not substantiated by either experimental or detailed observations of the germ cells during division. Nevertheless, a random sample of 150 individuals from my total collection shows a sex ratio of almost 1:1. The probability, with a 99.75 percent degree of assurance, that the ratio I have determined does not occur in nature is 0.05521.

SUMMARY

Part I

1. Cleavage in Micropterus salmoides salmoides Lac. is meroblastic discoidal. The blastodisc alone cleaves; the yolk remains unsegmented and is extra-embryonic.

2. A true discogastrula is formed; neither a segmentation cavity nor an archenteron develops. The cavities usually associated with them are replaced by the yolk. The yolk sphere is incorporated within the body of the embryo through epiboly of the germ ring, which corresponds to the definitive dorsal, ventral, and lateral lips of the primitive type of blastopore. Gastrulation is effected by cellular movement rather than by either delamination of the blastoderm or differential mitoses in the embryonic area of the germ ring. The dorsal lip remains in a fixed position relative to the oil globule.

3. The periblast nuclei are derived from the marginal cells of the blastoderm. They increase in number by mitotic division and migrate beneath the blastoderm to form the central periblast.

4. The blastoderm is the first tissue to be formed and the mesentoderm is the second tissue. The mesentoderm delaminates and produces mesoderm and entoderm. All of the entoderm is used in the formation of the alimentary tract. The major part of the mesoderm forms the muscle segments, and the remainder forms those structures usually classified as mesodermal in origin. The ectoderm arises directly from the blastoderm and consists of two portions; the epidermal stratum and the nervous stratum.

5. The gut and the kidney ducts are formed by a folding along the midline and by constriction of the somatic layer of the coelom,

respectively, as is typical in teleosts. The primary kidney ducts persist as the function excretory ducts, there being no secondary duct formation. No postanal gut is formed.

6. Kupffer's vesicle is homologous with the early lumen of the gut. It forms without a cellular floor, is converted into a tube in the same manner as the gut, and becomes the cloacal region of the alimentary tract.

7. The yolksac possesses a caudal subintestinal extension which is situated in the ventral mesentery between the gut and the subintestinal vein. This extension is produced by the backward growth of the tail.

Part II

1. Observations support an early segregation of the germ cells. The appearance of an excresence at the dorsal lip of the blastopore marks the time that they first can be recognized.

2. The potential primordial germ cells get into the periblast ventral to the dorsal lip and are shifted caudad in the caudal extension of the yolksac.

3. The primordial germ cells migrate by amoeboid movement. The path of migration is from the yolksac extension through the splanchnic mesoderm into the potential coelom, thence dorsad to the crest of the gut and through the mesentery and laterally adjacent mesoderm to the germ gland primordia.

4. The germ cells do not divide prior to reaching the region of the germ gland. Their apparent increase in number from one stage to the next is most likely due to their becoming more conspicuous as somatic differentiation progresses. The number of germ cells is more constant among individuals from the same egg mass than among those from different

egg masses.

5. The primordial germ cells possess no one special feature by which they can be identified. They are large cells with the general features which usually characterize primordial germ cells. Their magnitude is due to a retention of their original size as early blastomeres.

6. The germ cells are asymmetrically distributed to the gonad primordia, the right receiving the larger number. This is the result of a reduction in the potential migration area on the left side associated with the formation of the subintestinal vein.

7. No germ cells become located in the gonadal epithelium in the bass. They lie against but not in the epithelium, hence it is not germinal in nature. There is no transformation of stromal or epithelial cells into germ cells. The definitive sex cells are derived from the primordial germ cells only.

8. The presence of germ cells is apparently necessary for the formation of the germ gland. After its formation and prior to sex differentiation the germ gland passes through a long period of sexual indifference. During this time it increases in size through an increase in the amount of both germinal and stromal elements.

9. The indifferent gonad shows an anterioposterior gradation of development, the posterior portion developing earlier.

10. The gonads remain paired anteriorly but they fuse posteriorly. Each contains a gonocoel made patent by a separation of two layers of epithelium delaminated early in development. The gonocoels unite into a common cavity in the fused portions of the gonads and continue as a median oviduct or vas deferens.

11. Sex may be recognized at the 3.0-centimeter stage through the growth of the gonia in females.

12. The early ovary and testis differ from each other not only in structure but also in the number of germinal cells. The early ovary possesses a thick connective tissue tunic, which the testis lacks; the ovocoel is lined with flattened epithelial cells, whereas the testocoel is lined with low, irregular cuboidal cells; and the mesovarium is broad while the mesorchium is thin and delicate.

13. Sex may be recognized in gross dissection in the 4.0-centimeter stage. The ovaries are distended while the testes are thin strands. Both the ovaries and the testes are suspended along the ventral surface of the swimbladder.

14. The later ovary possesses lamellae which project into the ovocoel. Both the oöcytes and ova are in various stages of growth and yolk formation. The nucleus of the growing oöcyte loses its reticular structure and its chromatin collects into numerous rounded bodies peripherally arranged along the nuclear membrane.

15. The later testis is tubular. The spermatogonia lie in the walls of the tubules but do not form a part of their epithelium. Tubules are formed through outgrowths of the testocoel which penetrate the stroma between the spermatogonia so that the germ cells are left to lie around them. The testocoel corresponds to a primary collecting duct and its branches to secondary and tertiary ducts.

16. The sex ratio obtained indicates the presence of some sex determining factor, probably chromosomal.

LITERATURE CITED*

Abramowicz, Helene 1913 Die Entwicklung der Gonadenanlage und Entstehung der Gonocyten bei Triton taeniatus (Schneid). Morph. Jahrb., 47:593-644.

Agassiz, Alex. and C. O. Whitman 1884 On the development of some pelagic fish eggs. Preliminary notice. Proc. Am. Acad. Arts and Sci., 20:23-75.

Allen, B. M. 1904 The embryonic development of the ovary and testis of the Mammals. Am. J. Anat., 3:89-154.

------- 1906 The origin of the sex cells of Chrysemys. Anat. Anz., 29:217-236.

------- 1907a A statistical study of the sex cells of Chrysemys marginata. Anat. Anz., 30:391-399.

------- 1907b An important period of the sex cells in Rana pipiens. Anat. Anz., 31:339-347.

------- 1911a The origin of the sex cells of Amia and Lepidosteus. J. Morph., 22:1-36.

------- 1911b The origin of the sex cells in Necturus. Science N. Y. 33:268-269.

Bachmann, Freda M. 1914 The migration of the germ cells in Amiurus nebulosus. Biol. Bull., 26:351-366.

Balfour, F. M. 1877 The development of Elasmobranch fishes. J. Anat. Phys., 11:128.

------- 1881 A treatise on comparative embryology. Vol. II., xi + 655 p. Macmillan and Co., London.

Battle, Helen I. 1940 The embryology and larval development of the goldfish (Carassius auratus L.) from Lake Erie. Ohio Journ. Sci., 40(2):82-93.

------- 1944 The embryology of the Atlantic salmon (Salmo salar Linnaeus). Can. J. Res. D, 22:105-125.

Beard, J. 1893 Hermaphroditism of lampreys. J. Roy. Micr. Soc. London, 1893(2):156.

------- 1900 The morphological continuity of the germ cells in Raja batis. Anat. Anz., 18:465-485.

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*Italics have been supplied in some cases by the author.

Beard, J. 1902a The germ cells of Pristiurus. Anat. Anz., 21:50-61.

------- 1902b The numerical law of the germ cells. Anat. Anz., 21:189-200.

------- 1902c The germ cells. Part I. Raja batis. Zool. Jahrb., 16:615-702.

Beccari, N. 1922 Studî sulla prima origine delle cellule genitali nei Vertebrati. II. Ricerche nella Salamandrina perspicillata. Arch. Ital. Anat., supp., 18:29-95.

------- 1924 Studî sulla prima origine delle cellule genitale nei vertebrati. III. Ricerche nel Bufo viridis. Arch. Ital. Anat. Embry., 21(3):332-374.

Bennington, N. L. 1936 Germ cell origin and spermatogenesis in the Siamese fighting fish, Betta splendens. J. Morph., 60(1):103-126.

Berenberg-Gossler, H. von 1912 Die Urgeschlechtszellen des Huhnerembryos am 3 und 4 Bebrütungstage mit besonderer Berücksichtigung der Kern und Plasma strukturen. Arch. f. mikr. Anat., 81: 24-72.

------- 1914 Ueber Herkunft und Wesen der sogenannten primären Urgeschlechtszellen der Amnioten. Anat. Anz., 47:241-264.

Berg, Lec S. 1940 Classification of fishes both recent and fossil. Travaux l'Inst. Zool. Acad. Sci. URSS, 5(2):87-517. (Lithoprinted 1947, Edwards Bros., Ann Arbor)

Blocker, Hubert W. 1933 Embryonic history of the germ cells in Passer domesticus (L). Acta Zool., 14:111-152.

Böhi, U. 1904 Beiträge zur Entwicklungsgeschichte der Leibeshöle und der Genitalanlage bei Salmoniden. Morph. Jahrb., 32:505-586.

Bookhout, Cazlyn G. 1937 The germ cells cycle in the Guinea pig. I. The embryonic development of the testis. II. The postnatal development of the testis. Zeit. f. Zellforsch. mikr. Anat., 25(5):728-763.

Bouin, M. 1900 Ébauche génitale primordiale chez Rana temporaria. Bibliog. Anat., 8:103-108.

------- 1901 Histogenèse de la gland génitale femelle chez Rana temporaria (L). Arch. de Biol., 17:201-381.

Bounoure, L. 1924a Dérivés endodermiques dorsaux et premiére ébauche génitale chez les batraciens anoures. Compt. rend. Acad. Sci. Paris, 178:339-341.

Bounoure, L. 1924b Origine des gonocytes primaries chez les Urodèles et signification des ces éléments chez les amphibiens en général. Compt. rend. Acad. Sci. Paris, 179:1082-1084.

------- 1925 L'origine des gonocytes et l'évolution de la première ébauche génitale chez les batraciens. Ann. Sci. Nat. Zool., 8:201-278.

------- 1934 Recherches sur la lignée germinale chez la genouille rousse aux premier stades du développement. Ann. Sci. Nat. Zool., 17:67-248.

Brambell, F. W. R. 1927 The development and morphology of the gonads of the mouse. Part I. The Morphogenesis of the indifferent gonad and of the ovary. Proc. Roy. Soc. B, 101:391-409.

Breder, C. M. 1936 The reproductive habits of the North American sunfishes (Family Centrarchidae). Zoologica, N. Y., 21(1):1-48.

Burger, J. Wendell 1937 The continuity of the germ cells in the urodele Plethodon cinereus (Green). J. Morph., 60(2):489-514.

Burns, R. K. 1925 The sex of parabiotic twins in Amphibia. J. Exp. Zool., 42:31-90.

Butcher, E. O. 1927 The origin of the definitive ova in the white rat (Mus norvegicus albinus). Anat. Rec., 37:13-30.

------- 1929 The origin of the germ cells in the lake lamprey, Petromyzon marinus unicolor. Biol. Bull., 56:87-99.

Carr, Marjorie Harris 1942 The breeding habits, embryology and larval development of the Large-Mouthed Black Bass in Florida. Proc. New Eng. Zool. Club, 20:43-77.

Celestino da costa, A. 1932 Les gonocytes primares chez les gonocytes chez les Mammiferes (Guinea pig). Compt. rend. Assoc. Anat., 27:198-212.

Chambers, R. and C. G. Grand 1936 The chemotactic reaction of leucocytes to foreign substances in tissue culture. J. Cell and Comp. Physiol., 8:1-19.

Champy, C. 1913 Recherches sur la spermatogénèse des batrachiens et les éléments accessoires du testicule. Arch. zool. exp. et gen., 52:13-304.

Cheng, Tso-Hsin 1932a The germ cell history of Rana cantabrigensis Baird. I. Germ cell origin and gonad formation. Zeit. f. Zellforsch. mikr. Anat., 16:497-541.

------- 1932b The germ cell history of Rana cantabrigensis Baird. II. Sex differentiation and development. Zeit. f. Zellforsch. mikr. Anat., 16:542-596.

Christensen, K. 1930 Sex differentiation and development of the oviduct in Rana pipiens. Am. J. Anat., 45:159-187.

Cowperthwaite, M. H. 1925 Observations on pre- and postpubertal oogenesis in the white rat, Mus norvegicus albinus. Am. J. Anat., 36:69-89.

Cunningham, J. T. 1885a The significance of Kupffer's vesicle with remarks on other questions of vertebrate morphology. Quart. J. Micr. Sci., 97:1-14.

------- 1885b On the relations of the yolk to the gastrula in teleosteans and in other vertebrate types. Quart. J. Micr. Sci., 101:1-38.

------- 1887 On the structure and development of the reproductive elements in Myxine glutinosa. Quart. J. Micr. Sci., 27:49-76.

Dantschakoff, Wera 1931a Keimzelle und Gonade. I. A Von der entodermalen Wanterzelle bis zur Urkeimzelle in der Gonade. Zeit. Zellforsch. mikr. Anat., 13:448-510.

------- 1931b Keimzelle und Gonade. Die entodermale Wanderzelle als Stammzelle in der Keimbahn. Experimentelle Beweise. Zeit. Zellforsch. mikr. Anat., 14:376-384.

------- Wera Dantschakoff, Jr. and Lydia Bereskina 1931 I. Al Identität der Urkeimzellen und der entodermalen Wanderzellen. Experimentelle Beweise. Zeit. Zellforsch. mikr. Anat., 14:333-375.

------- 1935 Sur les facteurs déterminant l'emplacement des gonades chez le poulet. Compt. rend. Acad. Sci. (Paris), 200(17):1495-1497.

Dean, B. 1897 On a plan of development of a myxinoid. Science, 5:435.

Devillers, C. 1947 Explantations in vitro de blastodermes de poissons (Salmo, Esox). Experientia 3:71-72.

Dildine, Glenn C. 1936 Studies on teleostean reproduction. I. Embryonic hermaphroditism in Lebistes reticulatus. J. Morph., 60(1): 261-277.

Dodds, G. S. 1910 (On) The segregation of the germ cells of the teleost, Lophius. J. Morph., 21:563-612.

Dustin, A. P. 1907 Recerches sur l'origine des gonocytes chez les amphibiens. Arch. de Biol., 23:411-522.

------- 1910 L'origine et l'évolution des gonocytes chez les Reptiles (Chrysemys marginata). Arch. de Biol., 25:495-544.

Eigenmann, C. H. 1891 On the precocious segregation of the sex cells in Micrometrus aggregatus Gibbons. J. Morph., 5:481-492.

Eigenmann, C. H. 1896 Sex differentiation in the viviparous teleost Cymatogaster. Arch. Ent. Mech., 4:125-179.

Essenberg, J. M. 1923 Sex differentiation in the viviparous teleost Xiphophorus helleri Heckel. Biol. Bull., 45:46-97.

Everett, N. B. 1942 The origin of ova in the adult opossum. Anat. Rec., 82(1):77-91.

------- 1943 Observational and experimental evidences relating to the origin and differentiation of the definitive germ cells in mice. J. Exp. Zoöl., 92(1):49-91.

------- 1945 The present status of the germ cell problem in vertebrates. Biol. Rev., 20:45-55.

Fedorow, V. 1907 Über die Wanderung der Genitalzellen bei Salmo fario. Anat. Anz., 31:219-223.

Firket, J. 1914 Recherches sur l'organogénèse des glandes sexuelles chez les oiseaux. Arch. de Biol., 29:201-351.

------- 1920a Recherches sur l'organogénèse des glandes sexuelles chez les oiseaux. Arch. de Biol., 30:393-516.

------- 1920b On the origin of germ cells in higher vertebrates. Anat. Rec., 18:309-316.

Fischer, Isle 1935 Zur Keimbahn frage bei den Urodelen Amphibien. Jahr. Morph. Mikr. Anat. Abt. II, Zeit. Mikr. Anat. Forsch., 37(2):219-244.

Foley, James O. 1927 Observations on germ-cell origin in the adult male teleost, Umbra limi. Anat. Rec., 35(4):379-399.

Gatenby, J. B. 1916 The transition of peritoneal epithelial cells into germ cells in some amphibian anura, especially Rana temporaria. Quart. J. Micr. Sci., 61:275-300.

Gensch, Hugo 1882 Das secundäre Entoderm und die Blutbildung beim Ei der Knochenfische. Inaugural dissertation, Königsberg, 1882 (Not seen - quoted from Wilson 1889).

Goette, A. 1873 Der Keim des Forelleneies. Arch. f. mikr. Anat., 9: 679-708.

------- 1890 Entwicklungsgeschichte des Flussneunauges (Petromyzon fluviatilis). Abh. zur Ent. der Thiere Heft 5, Teil 1. 95 p. Hamburg u. Leipzig.

Goldsmith, J. B. 1928 The history of the germ cells in the domestic fowl. J. Morph. Physiol., 46:275-316.

Goldsmith, J. B. 1935 The primordial germ cells of the chick. I. The effect on the gonad of complete and partial removal of the "germinal crescent" and of removal of other parts of the blastodisc. J. Morph., 58(2):537-553.

Goodrich, H. B., J. E. Dee, C. M. Flynn and Rowena M. Mercer 1934 Germ cells and sex differentiation in Lebistes reticulatus. Biol. Bull., 67:83-96.

Grassi, B. 1919 Nuove ricerch sulla storia naturale dell' Anguilla. R. Com. Tal. Ital., 67:1-141.

Hall, R. W. 1904 The development of the mesonephros and the Müllerian ducts in Amphibia. Bull. Mus. Compt. Zool., 45:31-126.

Hamlett, G. W. D. 1935 Primordial germ cells in a 415 mm. human embryo. Anat. Rec., 61(3): 273-279.

Hann, H. W. 1927 The history of the germ cells of Cottus bairdii Girard. J. Morph. Physiol., 43:427-498.

Hargitt, G. T. 1924 Germ cell origin in the adult salamander, Diemyctylus viridescens. J. Morph. Physiol., 39:63-112.

------- 1925 The formation of the sex glands and germ cells of mammals. I. The origin of the germ cells in the albino rat. J. Morph. Physiol., 40:517-558.

Henneguy, Felix 1888 Recherches sur la développement des Poissons Osseux. Embryogenie de la Truite. J. de Anat. e Physiol., 24:1-183.

Heys, Florence 1931 The problem of the origin of germ cells. Quart. Rev. Biol., 6(1):1-45.

His, W. 1876 Untersuchungen über die Entwicklung von Knochenfischen, besonders über diejenige der Salmens. Zeit. Anat. u. Ent., 1: 1-40.

Hoadley, L. 1928 On the localization of developmental potencies in the embryo of Fundulus heteroclitus. J. Exp. Zool., 52:7-44.

Hoffmann, C. K. 1884 Zur Ontogenie der Knochenfische. Arch. f. mikr. Anat., 23:45-108.

------- 1886 Zur Entwicklungsgeschichte der Urogenitalorgane bei den Anamnia. Zeit. f. Wiss. Zool., 44:570-643.

------- 1893 Étude sur le développement de l'appareil urogenital des oiseaux. Verhl. Akad Wetensch. Amsterdam, Wiss. en. natuurkd. afd., II, 1 Nr.4,54.

Humphrey, R. R. 1925 The primordial germ cells of Hemidactylium and other Amphibia. J. Morph. Physiol., 41:1-43.

Jarvis, May 1908 The segregation of the germ cells of Phrynosoma cornutum. Preliminary note. Biol. Bull., 15:119-126.

Jordan, H. E. 1917 Embryonic history of the germ cells of the loggerhead turtle (Caretta caretta). Carnegie Inst. Wash., Pub. 251:313-344.

King, Helen D. 1908 The oogenesis of Bufo lentiginosus. J. Morph., 19:369-438.

Kingery, H. M. 1917 Oogenesis in the white mouse. J. Morph., 30:261-316.

Kingsbury, B. F. 1913 The morphogenesis of the mammalian ovary: Felis domestica. Am. J. Anat., 15:345-379.

Kingsley, J. S. and H. W. Conn 1883 Some observations on the embryology of the teleosts. Mem. Boston Soc. of Nat. History, 3:183-210.

Kirkham, W. B. 1919 The germ cell cycle in the mouse. Anat. Rec., 10: 217-219.

Kupffer, C. von 1868 Beobachtungen über die Entwicklung der Knochenfische. Arch. f. mikr. Anat., 4:209-272.

------- 1884 Die Gastrulation an den meroblastischen Eiern der Wirbelthiere und die Bedeutung des Primitive-streifs. Arch. f. Anat. u. Physiol. 1884:1-38.

Kuschakewitsch, S. 1910 Die Entwicklungsgeschichte der Keimdrüsen von Rana esculenta. Ein Beitrag zum Sexualitätsproblem. Fest. zum 60, Geburt. R. Hertwigs, 2:61-224.

Lamkin, J. Bayard 1901 The spawning habits of the large mouth black bass in the South. Trans. Amer. Fish. Soc., 1900(1901):129-131.

Lereboullet, M. 1854 Résumé d'un travail d'embryologie comparee sur le développement du Brochet, de la Perche et de l'Écrevisse. Ann. des Sci. Nat., 1:237-289, 2:39-80.

------- 1861 Recherches d'Embryologie comparee sur le développement de la Truite. Ann. des Sci. Nat. 4th Ser., 16:113-196.

Lewis, W. H. 1912a Experiments on localization in the eggs of a teleost fish (Fundulus heteroclitus). Anat. Rec., 6:1-16.

------- 1912b Experiments on localization and regeneration in the embryonic shield and germ ring of a teleost fish (Fundulus heteroclitus). Anat. Rec., 6:325-334.

Lubosch, W. 1903 Ueber die Geschlechtsdifferenzierung bei Ammocoetes. Anat. Anz., 23:66-74.

Luther, W. 1935 Entwicklungsphysiologische Untersuchungen am Forellenkeim: Die Rolle des Organisationzentrums bei der Entstehung der Embryonalanlage. Biol. Zbl., 55:114-137.

------- 1936a Potenzprüfungen an isolierten Teilstucken der Forellenkeimscheibe. Arch. Entwmech. Org., 135:359-383.

------- 1936b Austausch von präsumptiver Epidermis und Medullarplatte beim Forellenkeim. Arch. Entwmech. Org., 135:384-388.

------- 1937a Transplantations- und Defektversuche am Organisationzentrum der Forellenkeimscheibe. Arch. Entwmech. Org., 137: 404-424.

------- 1937b Ueber einen Fall von atypischer Organbildung beim Forellenkeim. Arch. Entwmech. Org., 137:425-434.

Lydell, Dwight 1904 The habits and culture of the black bass. Bull. U. S. Fish Comm., 22(1902):39-44.

MacLeod, J. 1881 Recherches sur la structure et la développement de l'appareil reproductive femelle des Téléostéens. Arch. de Biol., 2:497-532.

Maschkowziff, Alex 1934 Zur Phylogenie der Geschlechtsdrüsen und der Geschlechtsausführgänge bei den Vertebrata auf Grund von Forschungen betreffend die Entwicklung des Mesonephros und der Geschlechtsorgane bei den Acipenseridae, Salmoniden und Amphibien. I. Die Entwicklung des Mesonephros und der Genitaldrüse bei den Acipenseridae und Salmoidae. Zool. Jahrb. Abt. Anat. u. Ont., 59(1/2):1-68.

McCosh, Gladys K. 1930 The origin of the germ cells in Ambystoma maculatum. J. Morph., 50:569-611.

Mršić, W. 1923 Die Spätbefructung und derren Einfluss auf Entwicklung und Geschlechtsbildung, experimentell nachgeprüft an der Regenbogenforelle. Arch. f. mikr. Anat., 98:129-209.

------- 1930 Ueber das Auftreten intermediarer Stadium bei der Geschlechtsdifferenzierung der Forelle. Arch. Entwmech. Org., 123:301-332.

Müller, Johann W. A. A. 1875 Ueber das Urogenitalsystem des Amphioxus und der Cyclostomen. Jena. Zeit. Naturw., 9:94-129.

Nieuwkoop, P. D. 1946 Experimental investigations on the origin and determination of the germ cells and the development of the lateral plate and germ ridges in the Urodeles. Arch. Neerland. de Zool., 7:1-205.

Nussbaum, M. 1880 Zur Differenzierung der Geschlechter im Thierreich. Arch. f. mikr. Anat., 18:1-121.

Nussbaum, M. 1901 Zur Entwickelung des Geschlechts beim Huhn. Vehr. Anat. Ges., Vers., 15:38-40.

Obreshkove, V. 1924 Accessory testicular lobes in Diemyctylus viridescens, their probable origin and significance. J. Morph. Physiol., 39:1-46.

Odum, E. P. 1936 Notes on the history of the germ cells in the Toadfish, Opsanus tau. J. Elisha Mitchell Sci. Soc., 52(2):235-246.

Oellacher, J. 1872-73 Beiträge zur Entwicklungsgeschichte der Knochenfische, nach Beobachtungen am Bachforellenei. Zeit. f. Wiss. Zool., 22:373-421.

Okkelberg, P. O. 1921 The early history of the germ cells in the brook lamprey, Entosphenus wilderi (Gage), up to and including the period of sex differentiation. J. Morph., 35(1):1-152.

Oppenheimer, J. M. 1934 Experiments on early developing stages of Fundulus. Proc. Nat. Acad. Sci. Wash., 20:536-538.

------- 1935a Processes of localization in developing Fundulus. Proc. Nat. Acad. Sci. Wash., 21:551-553.

------- 1935b Localized vital staining of teleostean embryos. Science, 82:598.

------- 1936 Processes of localization in developing Fundulus. J. Exp. Zool., 73(3):405-444.

------- 1937 The normal stages of Fundulus heteroclitus. Anat. Rec., 68:1-15.

Pasteels, J. 1933 La gastrulation et la répartition des territoires dans la moitié dorsale du blastodisque de Truite (Salmo iridaeus). Compt. rend. Soc. de Biol., 113:425-428.

------- 1934a Répartition des territoires et mouvements morphogénétiques de la gastrulation de l'oeuf de truite ("Salmo iridaues")., Compt. rend. Soc. de Biol., 113:425-428.

------- 1934b Le role de l'activité mitotique dans la gastrulation et la formation de l'embryon chez les Téléostéens. Compt. rend. Soc. de Biol., 117:1231-1234.

------- 1936 Etudes sur la gastrulation des vertebres meroblastiques. I. Téléostéens. Arch. Biol., 47:205-308.

Perle, S. 1927 Origine de la première ébauche génitale chez Bufo vulgaris. Compt. rend. Acad. Sci. Paris, 184:303-304.

Politzer, G. 1933 Die Keimbahn des Menschen. Zeit. Ges. Anat. abt. I. Zeit. Anat. u. Entwicklungsges., 100(3):331-361.

Rabl, C. 1896 Über die Entwicklung des Urogenitalsystems der Selachier. Morph. Jahrb., 24:632-767.

Reagan, F. P. 1916 Some results and possibilities of early embryonic castration. Anat. Rec., 11:251-267.

Reighard, Jacob 1906 The breeding habits, development and propagation of the black bass. Bull. Mich. Fish. Comm., 7 (in 16th. bein. rep. state board Fish Comm. 1903-04).

Reinhard, Leonid 1924 Die Entwicklung des Parablasts und seine Bedeutung bei Telostiern nebst der Frage über die Entstehung der Urgeschlechtszellen. Arch. f. Mikr. Anat. u. Ent., 103(3/4): 339-356.

Richards, A. and J. T. Thompson 1921 The migration of the primary sex-cells of Fundulus heteroclitus. Biol. Bull., 40:325-348.

-------, H. R. Hulpieu, and J. B. Goldsmith 1926 A restudy of the germ-cell history in the fowl. Anat. Rec., 34:158.

Risley, Paul L. 1933 Contributions on the development of the reproductive system in Sternotherus odoratus (Latreille). I. The embryonic origin and migration of the primordial germ cells. Zeit. f. Zellforsch. u. mikr. Anat., 18:459-492.

------- 1934 The activity of the coelomic (germinal) epithelium of the male musk turtle Sternotherus odoratus (Latreille). J. Morph., 56(1):59-99.

Rückert, J. 1888 Über die Entstehung der Excretionsorgane bei Selachiern. Arch. Anat. u. Phys., Anat. Abt., Bd.2:205-278.

Rubaschkin, W. 1908a Über das erste Auftreten und Migration der Keimzellen bei Vogelembryonen. Anat. Hefte 35:241-261.

------- 1908b Zur Frage von der Entstehung der Keimzellen bei Saugetierembryonen. Anat. Anz., 32:222-224.

------- 1909 Über die Urgeschlechtszellen bei Saugetieren. Anat. Hefte, 39:603-652.

------- 1910a Über das erste Auftreten und Migration der Keimzellen bei Saugetierembryonen. Anat. Hefte, 41:243.

------- 1910b Chondriosomen und Differenzieurungsprocesse bei Saugetierembryonen. Anat. Hefte, 41:399-431.

Rugh, Roberts 1941 Experimental embryology (A manual of techniques and procedures). ix + 302 p., New York University Bookstore, New York.

Ryder, J. A. 1884 A contribution to the embryography of osseous fishes, with special reference to the development of the cod (Gaddus morrhua). Report of U. S. Fish Comm. 1882 (1884), 10:445-605.

Ryder, J. A. 1887 On the development of osseous fishes, including marine and fresh-water forms. Report of U. S. Fish Comm. 1885 (1887), 13:488-604.

Schapitz, Reinhold 1912 Die Urgeschlechtszellen von Amblystoma. Arch. f. mikr. Anat., Abt. II, 79:41-78.

Schwarz, Daniel 1889 Untersuchungen des Schwanzendes bei den Embryonen der Wirbelthiere. Nach Beobachtungen an Selachiern, Knochenfischen und Vögeln vergleichend dargestellt. Zeit. f. Wiss. Zool., 49:191-223.

Semon, R. 1887 Die indifferente Anlage der Keimdrüsen beim Hühnchen und ihre Differenzierung zum Hoden. Jena Zeit. Med. Naturwiss., 21:46-86.

------- 1891 Studien über den Bauplan des Urogenitalsystems der Wirbeltiere. Dargelegt an der Entwicklung dieses Organsystems bei Ichthyophis glutinosus. Jena Zeit. Med. Naturwiss., 26:

Semper, C. G. 1875 Das Urinogenitalsystem der Plagiostomen und seine Bedeutung für der übrigen Wirbeltiere. Arbt. Zool. Inst. Würzburg, 1875, 2:195-509.

Simkins, C. S. 1923 On the origin and migration of the so-called primordial germ cells in the mouse and the rat. Acta Zool., 4(2/3): 241-284.

Sink, E. W. 1912 The origin of the germ cells in the toadfish (Opsanus tau). Mich. Acad. Sci. Rep., 14:212-215.

Solberg, Archie N. 1939 The development of a bony fish. Prog. Fish-Cult., 40:1-19.

Spehl, G. and J. Polus 1910 Les premiers stades du développement des glandes génitales chez l'axolotl. Arch. de Biol., 27:63-90.

Swaen, A. and A. Brachet 1899 Étude sur les premières phases du développement des organes dérivés du mésoblaste chez les poissons téléostéens. Arch. de Biol., 16(2):173-303, 304-311.

------- 1901 Deuxieme partie. Étude sur les premières phases du développement des organes dérivés du mesoblaste chez les poissons téléostéens. Arch. de Biol., 18:73-190.

Swift, Charles H. 1914 Origin and early history of the primordial germ-cells in the chick. Am. J. Anat., 15:483-516.

------- 1916 Origin of sex-cords and spermatogonia in male chicks. Am. J. Anat., 20:373-410.

Swingle, W. W. 1921 The germ cells of anurans. I. The male sexual cycle of Rana catesbeiana (Larvae). J. Exp. Zool., 32:235-237.

Swingle, W. W. 1926 The germ cells of anurans. II. An embryological study of sex differentiation in Rana catesbeiana. J. Morph., 41(2):441-546.

Trabucco, Armando 1938 Origin y evolution de la cellula germinal primaria. Semana Med., 48(2325):261-271.

Tschaschkin, S. 1910 Ueber die Chondriosomen der Urgeschlechtszellen bei Vogelembryonen. Anat. Anz., 37:597-607, 621-631.

Vanneman, A. S. 1917 The early history of the germ cells in the armadillo, Tatusia novemcincta. Am. J. Anat., 22:341-364.

Van Oordt, G. J. 1924 The relation between the development of the secondary sex characters and the structure of the testis in the teleost Xiphophorus helleri. Brit. J. Exp. Biol., 3:43-59.

Waldeyer, W. 1870 Eierstock und Ei. Leipzig.

Wheeler, W. M. 1899 The development of the urinogenital organs of the lamprey. Zool. Jahrb. Abt. für Anat., 13:1-88.

Wijhe, J. W. van 1889 Über die Mesodermsegmente des Rumpfes und die Entwicklung des Excretionssystems bei Selachiern. Arch. f. mikr. Anat., 33:461-516.

Winge, O. 1922 A peculiar mode of inheritance and its cytological explanation. J. Gen., 12:137-144.

Wilson, Henry V. 1891 The embryology of the sea bass, Serranus atrarius. Bull. Us. Fish Comm. (1889), 9:209-278.

Winiwarter, H. von and G. Sainmont 1909 Nouvells recherches sur l'ovogeneses et l'organogénèse de l'ovaire des Mammiferes (chat). Arch. de Biol., 24:1-142, 165-276, 373-431, 627-650.

Witschi, Emil 1914 Experimentelle Untersuchungen über die Entwicklungsgeschichte der Keimdrüsen von Rana temporaria. Arch. f. mikr. Anat., 85 and 86:9-113.

------- 1929 Studies on sex differentiation and sex determination in amphibians. I. Development and sexual differentiation of the gonads of Rana sylvatica. J. Exp. Zool., 52:235-266.

------- 1932 In "Sex and Internal Secretions," edited by Edgar Allen Chap. 5, 160-245, Williams and Wilkins Bo., Baltimore.

------- 1935 Die Amphisexualitat der Embryonalen Keimdrüse des Hausperlings, Passer domesticus L. Biol. Centralblatt, 55(3/4):168-174.

------- 1948 Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. Carnegie Inst. Wash. Pub., 575, Contributions to Embryology, 32:67-80.

Woodger, J. H. 1925 Observations on the origin of the germ cells of the fowl, Gallus domesticus, studied by means of their Golgi bodies. Quart. J. Micr. Sci., 69(3):445-462.

Woods, F. A. 1902 Origin and migration of the germ cells in Acanthias. Am. J. Anat., 1:307-320.

Wolf, L. E. 1931 The history of the germ cells in the teleost, Platypoecilus maculatus. J. Morph., 52:115-154.

Ziegler, H. E. 1887 Die Entstehung des Blutes bei Knochenfischembryonen. Arch. f. mikr. Anat., 30:596-665.

------- 1896 Die Entstehung des Periblastes bei den Knochenfischen. Anat. Anz., 12:353-370.

------- 1902 Lehrbuch der vergleichenden Entwicklungsgeschichte der niederen Wirbeltiere. xii + 366 p., 1 pl. Fischer, Jena.

EXPLANATION OF PLATES

Abbreviations

AP - anterior pole
bd - blastoderm
ch - chorion
Coe - coelom and coelomic mesoderm
DA - dorsal aorta
DL - dorsal lip of the blastopore
DM - dorsal mesentery
EEE - extra-embryonic ectoderm (area)
eps - epidermal stratum
ES - embryonic shield
G - gut
gc - germ cell
GR - germ ring
gs - genital sinus
HE - head end
KVC - Kupffer's vesicle
LL - lateral lip of the blastopore
Med - mesentoderm
N - neural tube
NC - notochord
NCM - nephridiocoelomic mesoderm
OD - oviduct
OG - oilglobule
OVC - ovocoel
PND - pronephric ducts
PP - posterior pole
PPR - pre-periblastic ridge
S - swim bladder
SIV - subintestinal vein
SOM - somite
tc - testocoel
td - testicular duct
ub - urinary bladder
VL - ventral lip
Y - yolk
yp - yolk plug
YSE - yolksac extension

Plate 1

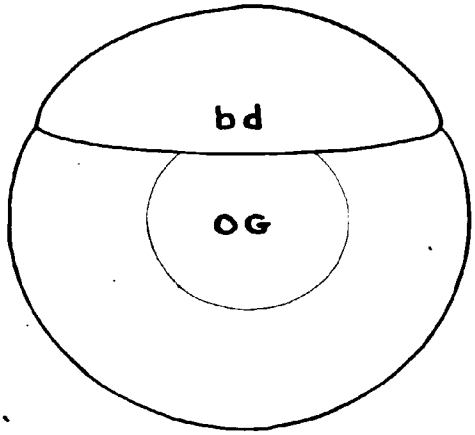
Explanation of Figures
Figures 1-4 not drawn to scale

1 Outline drawing of the blastodisc (bd) showing the relationship with the oil globule (og).

2 Outline drawing of the two-cell stage. Note that the cleavage furrow does not extend into the yolk.

3 Drawing of the ten- to eleven-hour stage, viewed from the side as an opaque sphere. Blastoderm has spread down over the yolk sphere as far as the equator. Dotted line represents the advance of the periblast before the spreading blastoderm.

4 Drawing of the ten- to eleven-hour stage, viewed posteriorly.



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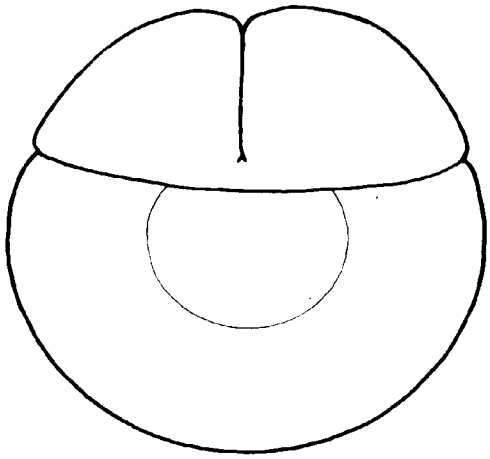

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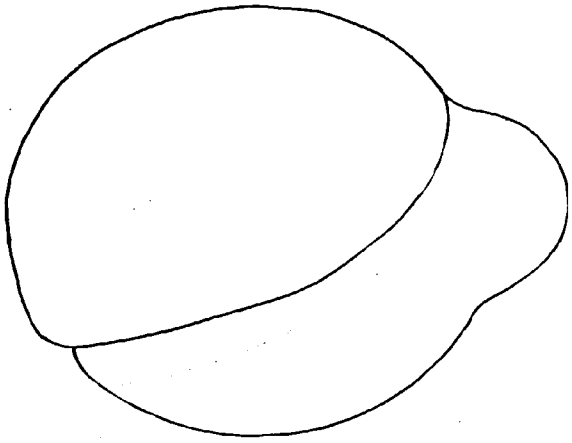

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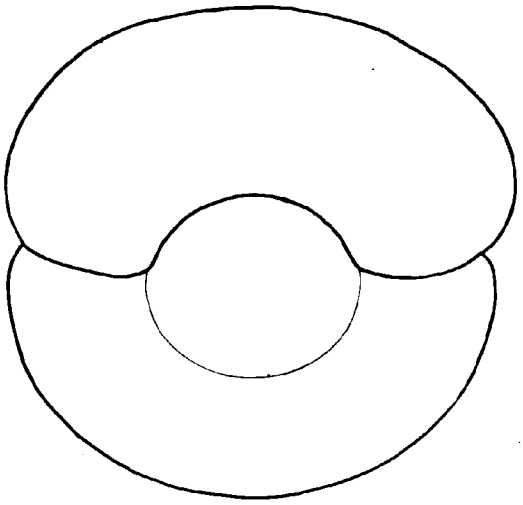

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Plate 2

Explanation of Figures
Figures 5-9 not drawn to scale

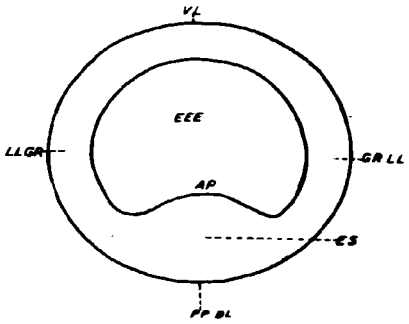
5 Diagram of the early germ ring (6-8 hours of development), formed as a result of the eccentric thinning and flattening of the blastoderm.

6 Diagram of the late germ ring (about 12 hours of development), showing the anterior growth of the embryonic shield (es) across the extra-embryonic region (eee).

7 Outline drawing of the four-hour blastoderm and the pre-periblastic ridge (ppr).

8 Diagrammatic representation of gut formation. See text for explanation.

9 Diagrammatic representation of a cross section of a forty-four-hour embryo. Note that the coelomic mesoderm (coe) does not extend down and around the yolk (y). The dotted lines represent subsequent extensions of the coelomic layers.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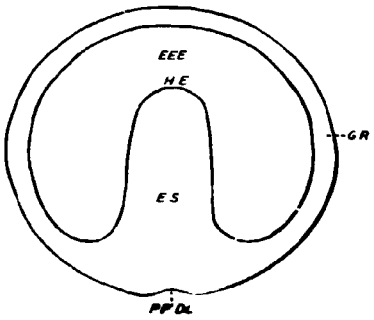
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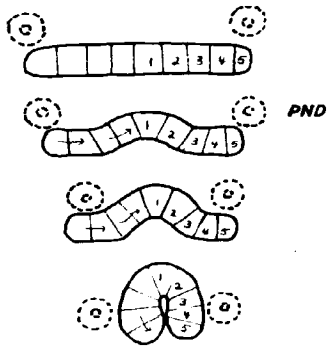
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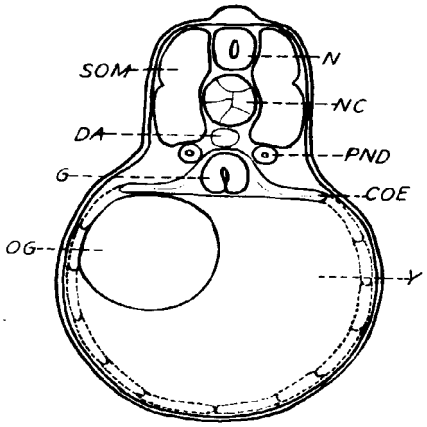
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Plate 3

Explanation of Figures
Figures are unretouched photomicrographs

10 Section of the early gastrula with the chorion (ch). Approximately 160 X.

11 Detail of several cells of the four-hour blastoderm. Observe that every cell shown is preparing to divide. Note also the granular cytoplasm. Approximately 920 X.

12 Detail of several newly-formed periblastic nuclei in periblastic ridge of a four-hour blastoderm. These nuclei are in the process of spindle formation. Approximately 920 X.

13 Section through the dorsal lip of an early gastrula (8-9 hours of development) showing the formation of the mesentoderm. Note the absence of mitotic figures. Approximately 160 X.

10

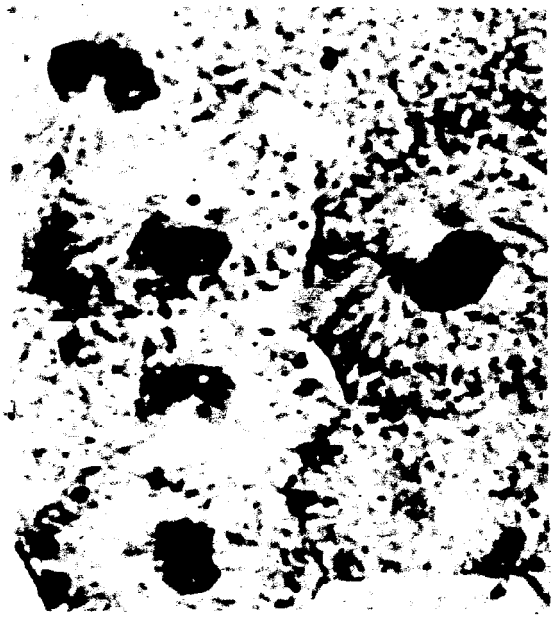

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Plate 4

Explanation of Figures
Figures are unretouched photomicrographs

14 Sagittal section of a fourteen-hour gastrula. The ventral lip has grown epibolically around the yolk from about point (x) to point (y) to form the yolk plug (yp) consisting of yolk and the oil globule. The oil globule is represented by the somewhat circular space ventral to the dorsal lip. Approximately 40 X.

15 Sagittal section of the embryonic shield of a fourteen-hour embryo showing the extent of the mesentoderm. Approximately 80 X.

14

15

Plate 5

Explanation of Figures
Figures are unretouched photomicrographs

16 An almost midsagittal section of the excresence of potential germ cells at the dorsal lip. The cells of the excresence give the appearance of being rolled up. Observe the presence of two and possibly three germ cells between the dorsal lip and the periblast. Approximately 540 X.

17 A parasagittal section of the late gastrula showing the formation of the excresence of potential germ cells at the dorsal lip. Note the germ cell (gc) just within the dorsal lip. Also note the striking similarity between the nucleus of the germ cell and that of cell "a" of the excresence. Approximately 540 X.

18 An enlargement of the dorsal lip region of the gastrula showing the area of invagination. Observe that the epidermal stratum does not invaginate. Approximately 600 X.

16

17

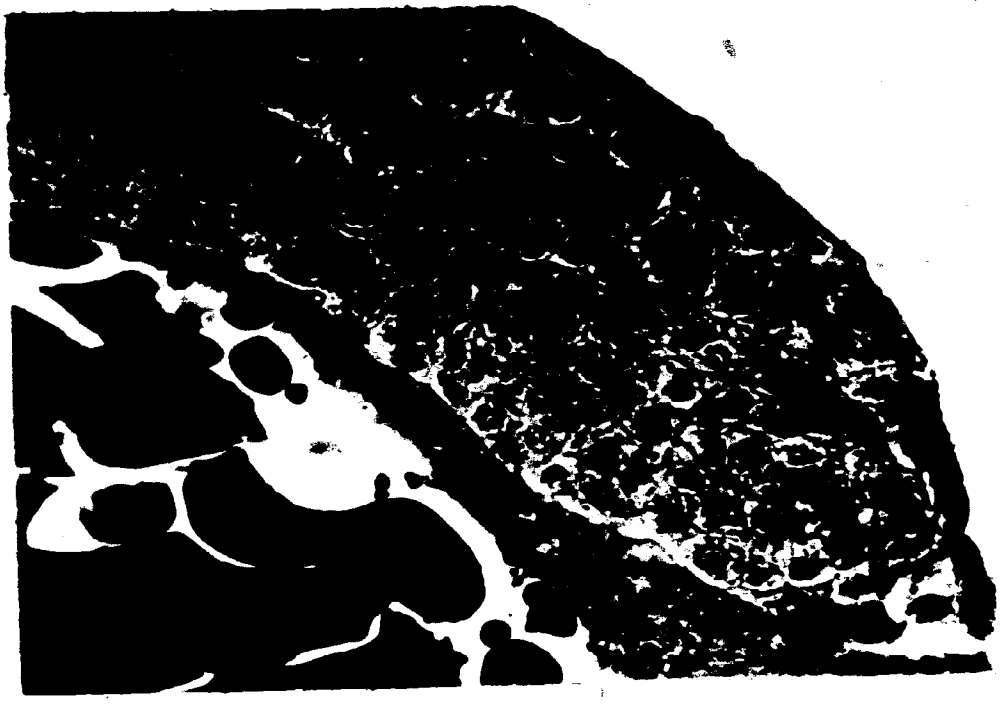

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Plate 6

Explanation of Figures
Figures are unretouched photomicrographs

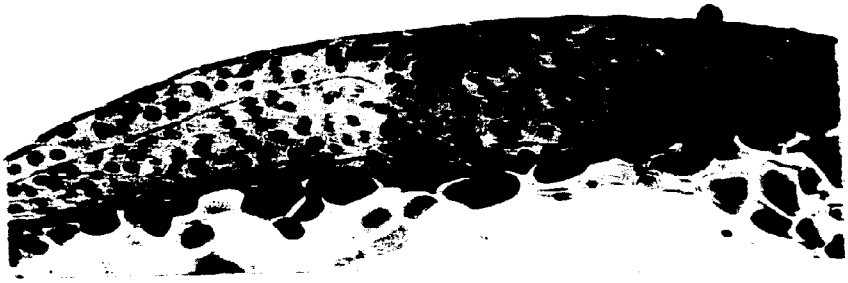
19 Cross section through a twelve- to fourteen-hour embryo showing the first organization of the mesentoderm into the somites and the notochordal anlage. At this stage the nervous ectoderm is thicker laterally than mesially. Approximately 150 X.

20 Cross section of a sixteen- to eighteen-hour embryo. The notochord is beginning to round up, the somites are thicker mesially, and the nervous ectoderm is beginning to shift mesiad. The entoderm is just visible as a unicellular lamella lying ventral to the somites (see also fig. 24). Approximately 150 X.

21 Cross section of a twenty- to twenty-two-hour embryo. The nerve wedge rests on the rounded notochord and is bordered laterally by the dorsally arching somites. The entoderm, although indistinct in this figure, extends from about point (x) to point (y). Approximately 200 X.

22 Cross section through the midtrunk region of a thirty-two-hour embryo showing a further differentiation of the somites, notochord, gut, and nerve wedge. Approximately 250 X.

19



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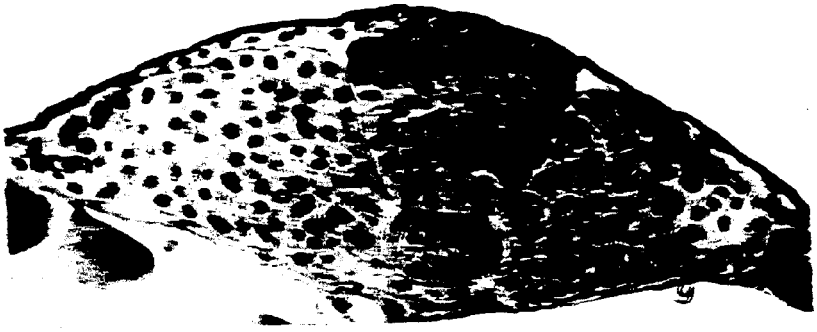

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Plate 7

Explanation of Figures
Figures are unretouched photomicrographs

23 A section through the lateral limits of the embryonic shield. The ectoderm continues over the yolk while the mesentoderm does not extend beyond the lateral limits of the shield (x). The entoderm extends to point (y). Approximately 300 X.

24 A section through the midtrunk region of a sixteen- to eighteen-hour embryo. The characteristic flattened condition of the epidermal stratum is very well demonstrated. The entoderm appears as a thin lamella ventral to the notochord and the somites. Several periblastic nuclei may be seen in the periblast immediately below the notochord. Approximately 800 X.

23

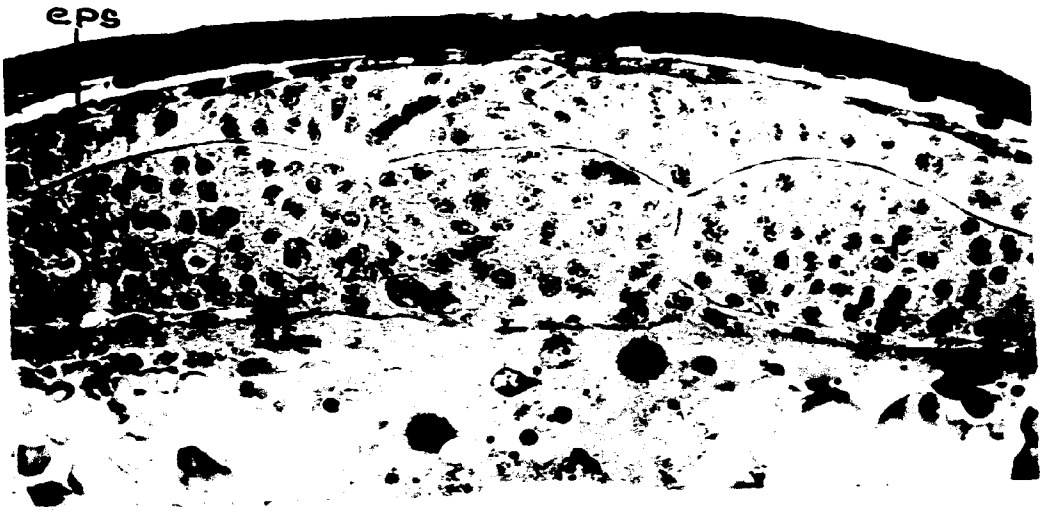

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Plate 8

Explanation of Figures
Figures are unretouched photomicrographs

25 A section through the hindgut region of a thirty-two-hour embryo. Three germ cells (1, 2 and 3) are lying in the periblast. Approximately 250 X.

26 Section through a forty-one-hour embryo at the level of junction of the yolksac extension. A germ cell is in the mesoderm just to the left of the gut. Approximately 250 X.

27 A portion of the periblast ventral to Kupffer's vesicle in a twenty-two-hour embryo. A germ cell (gc) may be seen lying in the periblast ventral to the cells of the vesicle (KVC). Note that the outline of the germ cell is very distinct. Approximately 1500 X.

28 An enlargement of the germ cells shown in figure 25 showing their definite cellular outline. Approximately 1500 X.

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Plate 9

Explanation of Figures
Figures are unretouched photomicrographs

29 A transverse section through a thirty-seven-hour embryo at the level of junction of the yolksac extension. Two germ cells (gc) are seen in the process of migration. Approximately 720 X.

30 A section through the posterior trunk region and the yolksac extension of a forty-four-hour embryo. A primordial germ cell is seen ventral to the left pronephric duct. The large space within the ventral mesentery was once filled with periblastic yolk, which was withdrawn prior to fixation. Observe the formation of the subintestinal vein ventrally where the two mesenteric limbs meet along the midline. Approximately 600 X.

29

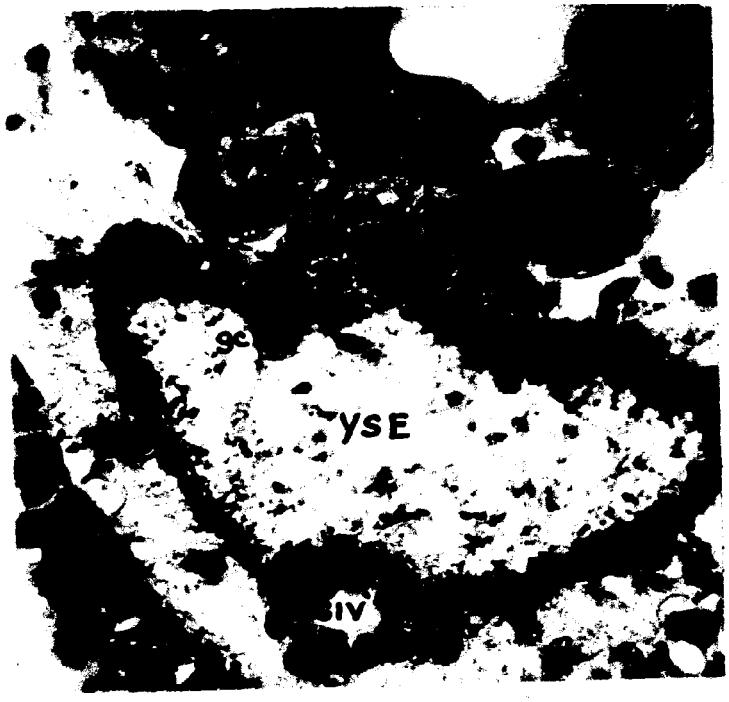

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Plate 10

Explanation of Figures
Figures are unretouched photomicrographs

31 A higher magnification of the pronephric duct region of figure 30. Observe the granular cytoplasm and diffuse arrangement of the chromatin in the nucleus in the primordial germ cell (gc). Close observation will disclose that the germ cell possesses a blunt pseudopodial extension which is directed dorsally toward a somatic cell nucleus (a). Approximately 1760 X.

32 A higher magnification of the primordial germ cell of figure 31. The pseudopodium of the germ cell extends almost to the nucleus above. Its right-hand margin passes over the dark mass to the left; and its left-hand margin passes just to the right of the somatic nucleus on the left (a). Observe the difference in chromatin arrangement in the nucleus of the primordial cell as compared with that of the nuclei of the somatic cells. Approximately 8000 X.

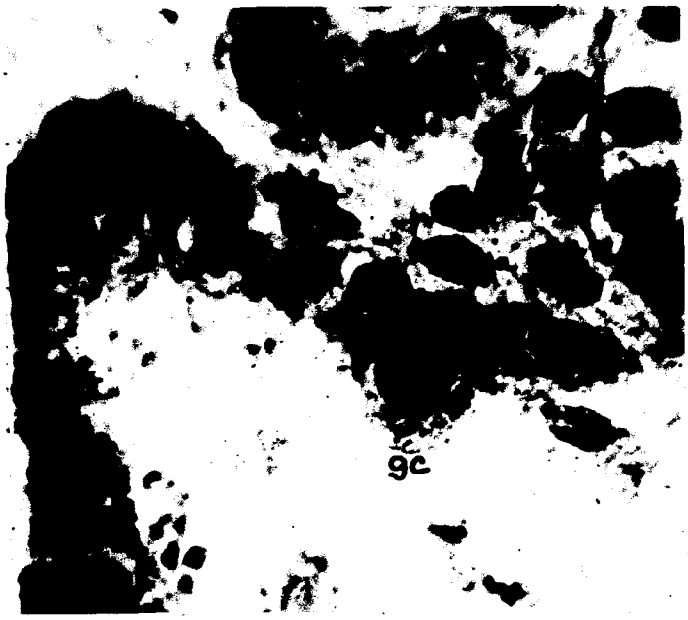

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Plate 11

Explanation of Figures
Figures are unretouched photomicrographs
Figures 33-35 are from the same embryo

33 A transverse section through the posterior trunk region of a forty-four-hour embryo. Two germ cells (gc 1 and gc 2) may be seen lying in the mesoderm to the right and left of the gut. Approximately 600 X.

34 A section taken posterior to that shown in figure 33. A germ cell is seen lying in the yolksac extension space ventral to the right pronephric duct. This cell possesses an attraction sphere. Observe the formation of the subintestinal vein ventrally. Approximately 600 X.

35 A section taken posterior to that shown in figure 34 and almost to the posterior limit of the yolksac extension shows a germ cell lying in the ventral mesentery. Note the similarity of its nucleus with that of previously demonstrated germ cells. Observe how definite its outline is as contrasted with those of somatic cells. Approximately 600 X.

36 A transverse section through the posterior trunk region of a 3.5-millimeter larva. Note the relative size of the yolksac extension (yse) and compare with figure 30. Approximately 340 X.

33

34

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36

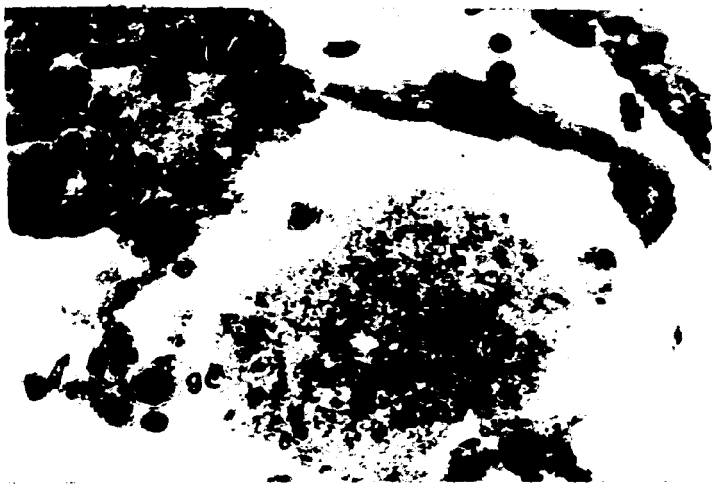
Plate 12

Explanation of Figures
Figures are unretouched photomicrographs

37 A cross section through the yolksac extension of a forty-five-hour embryo. During preparation the mesentery was slightly torn. A primordial germ cell (gc) may be seen lying next to a hemocytoblast. Approximately 480 X.

38 Is an enlargement of the region adjacent to the germ cell. Observe that the cytoplasm of the germ cell is arranged into a "cap" around the nucleus. The germ cell also possesses an attraction sphere. Approximately 1300 X.

39 A cross section through the yolksac extension of a forty-five-hour embryo. Two germ cells (1 and 2) may be seen lying in the space ventral to the gut. Germ cell 1 possesses an attraction sphere. Approximately 960 X.



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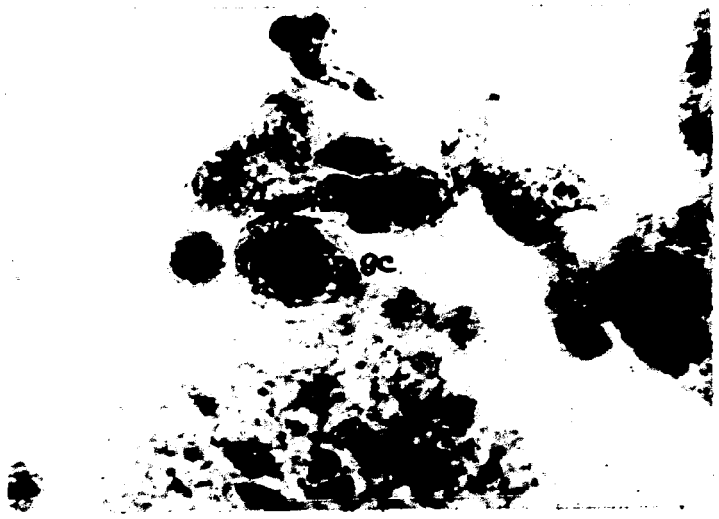

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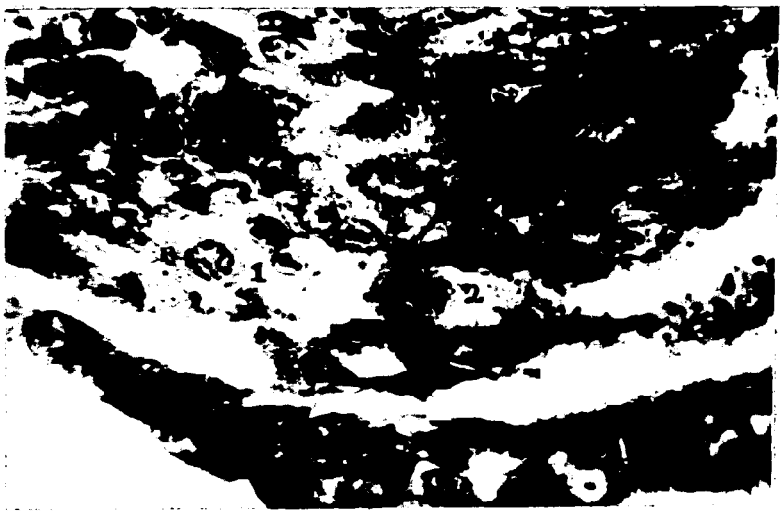

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Plate 13

Explanation of Figures
Figures are unretouched photomicrographs

40 A transverse section through the posterior trunk region of a forty-seven-hour embryo. Two germ cells are seen lying in the potential coelomic space to the left of the gut. Approximately 360 X.

41 A transverse section through the posterior trunk region of a forty-seven-hour embryo, apparently less developed than that shown in figure 40. Two germ cells are present in the mesoderm to the right and left of the gut. Approximately 250 X.

42 A cross section through the hindgut region of a 3.5-millimeter larva 0-1 days post hatching. Four germ cells may be seen, apparently lying, in the potential coelomic cavity. Careful observation will disclose that the two germ cells on the right are bordered both medially and laterally by flattened mesodermal cells. Approximately 500 X.

43 A cross section of the hindgut region of a 3.5-millimeter larva. A germ cell may be seen at the crest of the mesentery dorsal to the gut. This cell appears to be passing through the splanchnic layer of the mesentery. The space (coe) to the left of this cell marks the beginning of the coelom, posteriorly. Approximately 500 X.

40

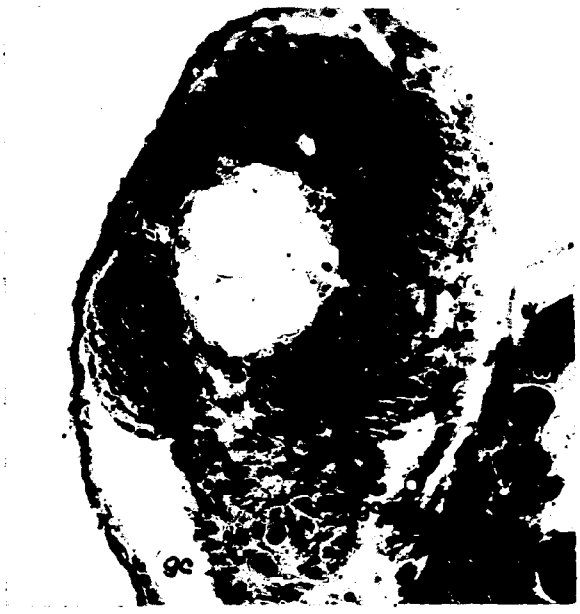

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Plate 14

Explanation of Figures
Figures are unretouched photomicrographs

44 A transverse section through the hindgut region of a 4.0-millimeter larva. Five germ cells (1-5) lie in the dorsal mesentery dorsal to the gut. Approximately 880 X.

45 A longitudinal section of the hindgut region of a 4.5-millimeter larva. Four germ cells (1-4) may be seen lying dorsal to the gut (g) and ventral to the pronephric duct (pnd). These cells are now retroperitoneal. Approximately 500 X.

44

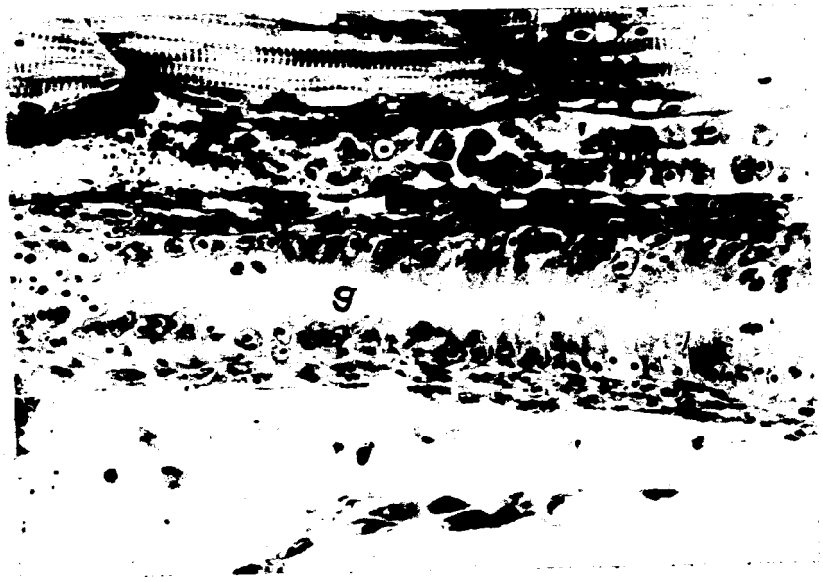

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Plate 15

Explanation of Figures
Figures are unretouched photomicrographs

46 A transverse section through the hindgut region of a 5.0-millimeter larva two days post hatching. Three germ cells may be seen lying dorsal to the mesentery. The coelomic spaces are very well defined and are lined with a delicate mesothelium. Approximately 800 X.

47 A transverse section through the hindgut region of a 6.5-millimeter larva. Four germ cells (1-4) lie retroperitoneally; two ventral to the right and two ventral to the left pronephric ducts. The beginning of the lateral shifting of the germ cells may be observed in this section. Approximately 800 X.

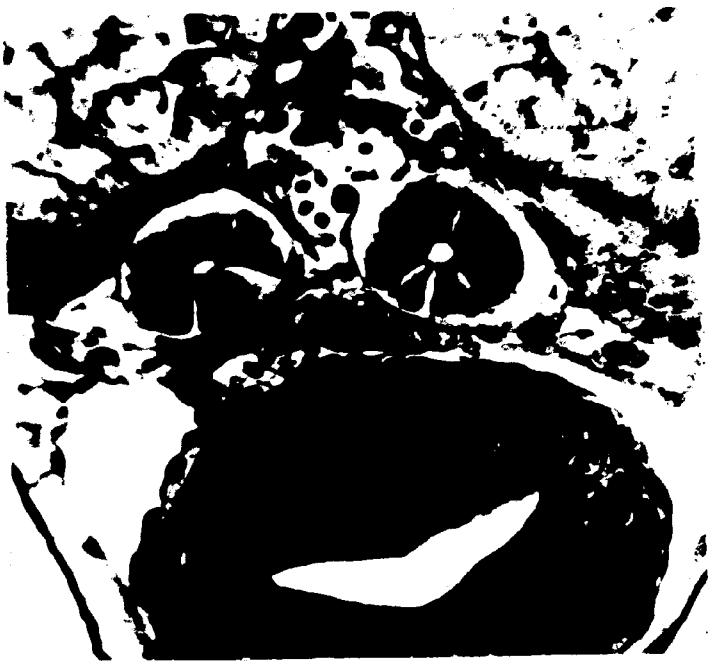

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Plate 16

Explanation of Figures
Figures are unretouched photomicrographs

48 A cross section through the hindgut region of a 6.5-millimeter fry. A germ cell (gc) may be seen lying ventral to the right pronephric duct. The germ gland later forms in this immediate region. Approximately 680 X.

49 A transverse section through the hindgut region of an 8.0-millimeter fry. Two germ cells are lying in the germ gland primordia. Note how thin and delicate the dorsal mesentery (dm) is and compare with earlier stages. Approximately 480 X.

50 A transverse section through the hindgut region of an 8.0-millimeter fry. Two germ cells lie in the left germ gland primordium. Approximately 480 X.

51 A section of the germ gland of an 8.0-millimeter fry. The gonium is surrounded by a single layer of epithelial cells. Approximately 500 X.

48

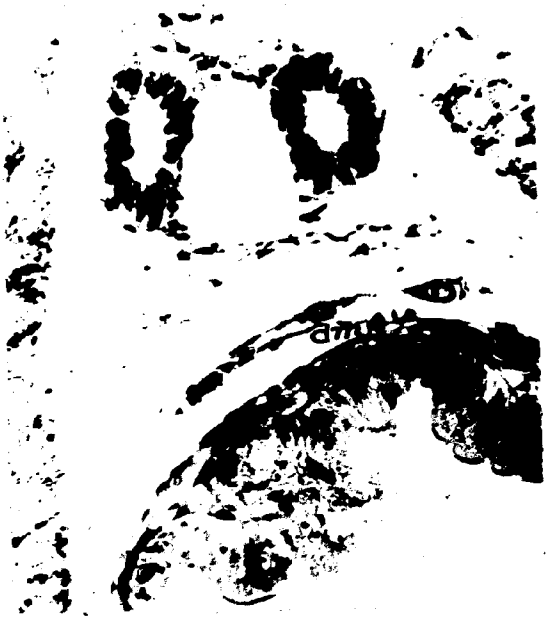

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Plate 17

Explanation of Figures
Figures are unretouched photomicrographs

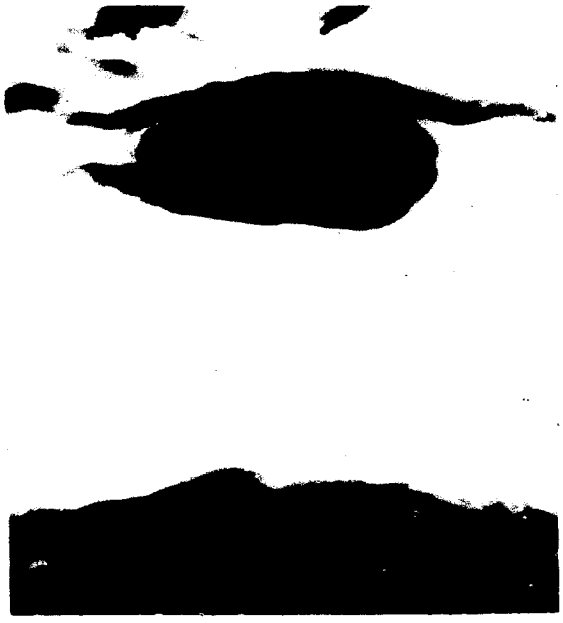
52 A section of the germ gland region of a 10.0-millimeter fry showing a germ cell (gonium) being invested by a second layer of epithelial cells. It appears that these cells are migrating down and around the gonium. Approximately 1000 X.

53 A transverse section of the germ gland of an 11.0-millimeter fry. Two layers of epithelial cells overlie the gonium. The inner layer may be likened to a follicular layer and the outer to a true epithelial layer. Approximately 1000 X.

54 A section through the germ gland of an 11.0-millimeter fry. The gland hangs suspended in the coelom by a delicate mesentery. Approximately 320 X.

55 A cross section through the germ gland of an 18.0- to 20.0-millimeter fry-fingerling. A separation of the epithelial layers has occurred forming a space between them. The gonium is contained within the inner layer. The definitive adult relationship between the gonad and the swimbladder (s) is established by this stage. Approximately 400 X.

52



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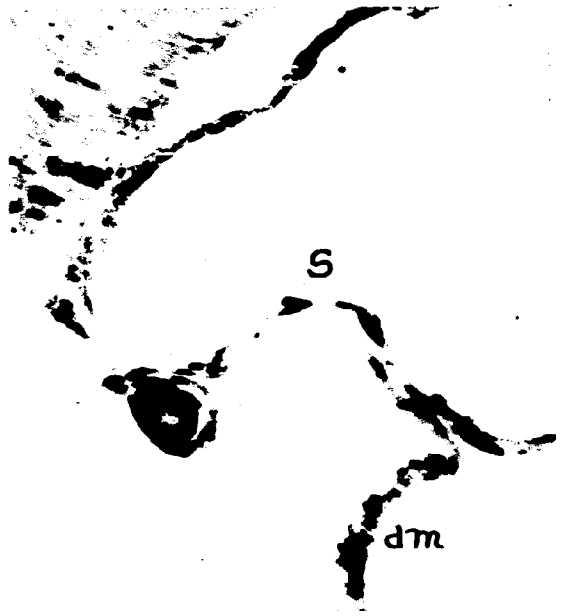

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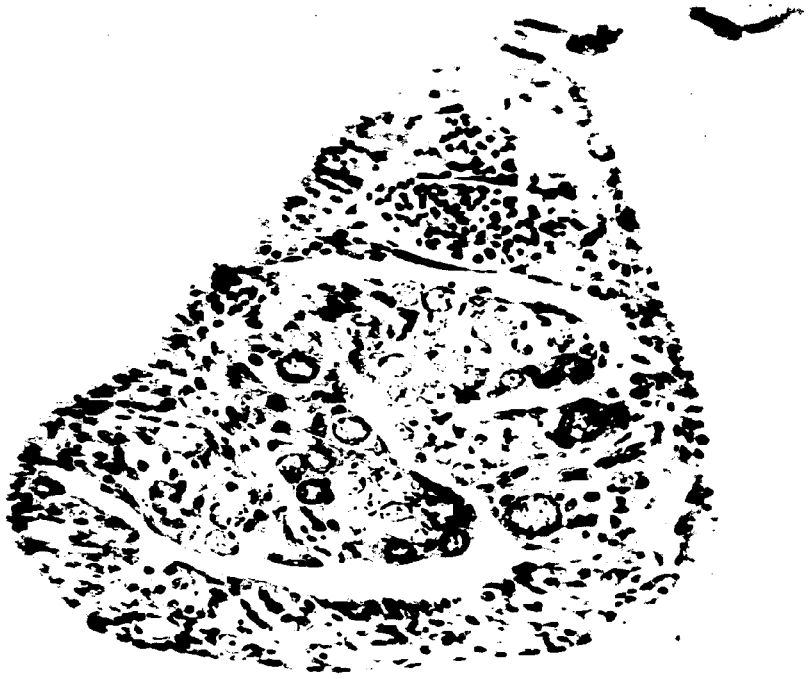
Plate 18

Explanation of Figures
Figures are unretouched photomicrographs

56 A transverse section of the late indifferent gonad. Note the predominance of stroma. Approximately 400 X.

57 A cross section of the right and left ovaries of a sexually differentiated female 3.0 centimeters in length. Note the thickness of the connective tissue tunic and the broadness of the mesentery. Approximately 250 X.

58 A cross section of the fused ovaries of a 3.0-centimeter female. Approximately 250 X.



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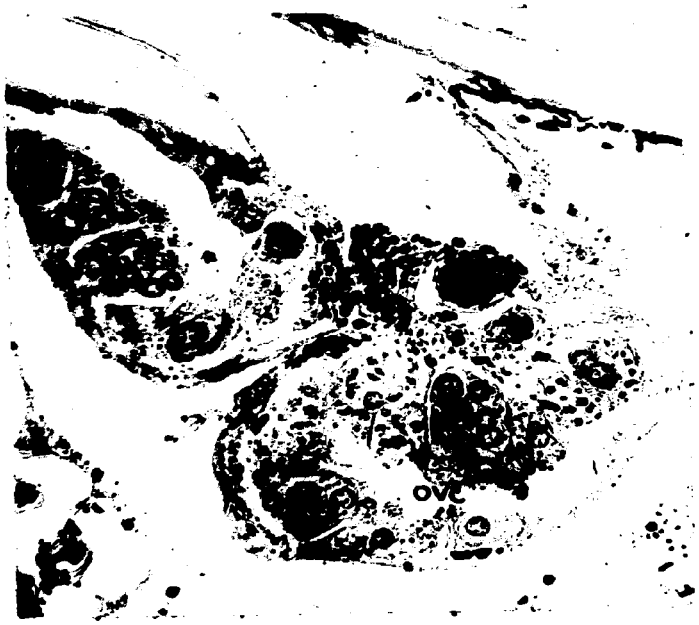

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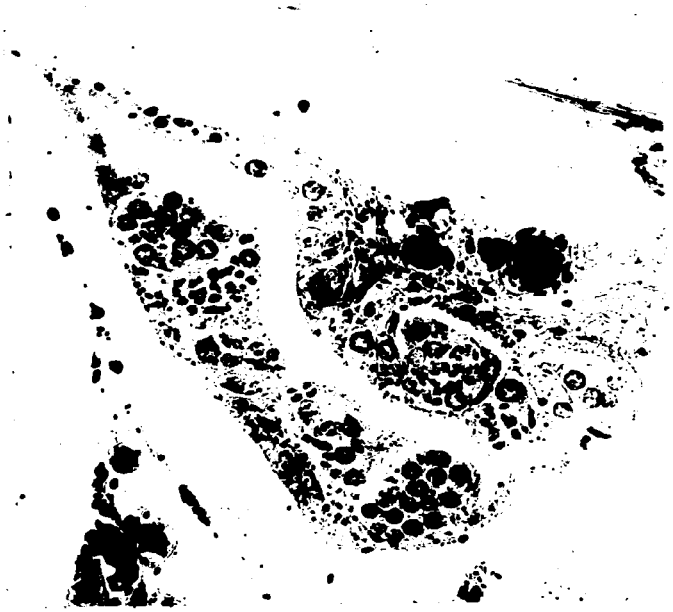

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Plate 19

Explanation of Figures
Figures are unretouched photomicrographs
Figures 59-60 are from same embryo

59 A section through the sinus formed by the fused ovocoels of the ovaries. Note that it lies in the mesentery dorsal to the gut. Both a dorsal and ventral portion of the dorsal mesentery may be seen (a and b). Approximately 250 X.

60 A section taken posterior to that shown in figure 59 shows the relationship of the oviduct with the urinary bladder above (ub) and the gut below. Approximately 250 X.

61 A transverse section through the ovary of a 3.0-centimeter fingerling. Observe that the oöcytes are contained within lamellae which project into the ovocoel. Approximately 120 X.

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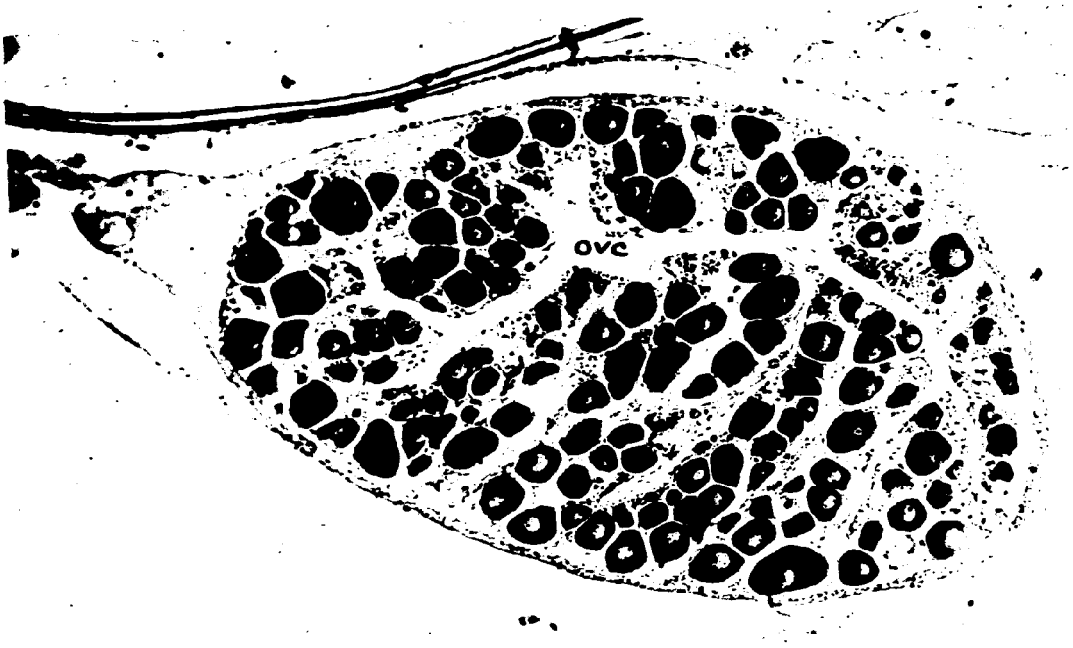

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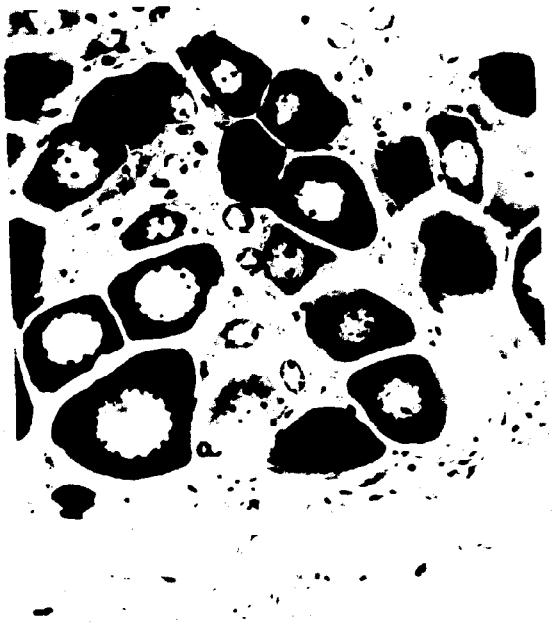
Plate 20

Explanation of Figures
Figures are unretouched photomicrographs

62 a section through the ovary of a 4.5-centimeter fingerling. Note the arrangement of the chromatin along the nuclear membrane in the larger oöcytes. Note also the two regions, light and dark, of the cytoplasm in oöcyte (a). Approximately 250 X.

63 A section through the ovary of a 3.5-centimeter fingerling. Note the oöcytes in various stages of growth, ranging all the way from oögonia to advanced stages. Approximately 680 X.

64 A section through the ovary of a 3.5-centimeter fingerling showing the cytoplasmic differentiation in the rapidly growing oöcytes. This is particularly good in oöcyte (a). Approximately 340 X.



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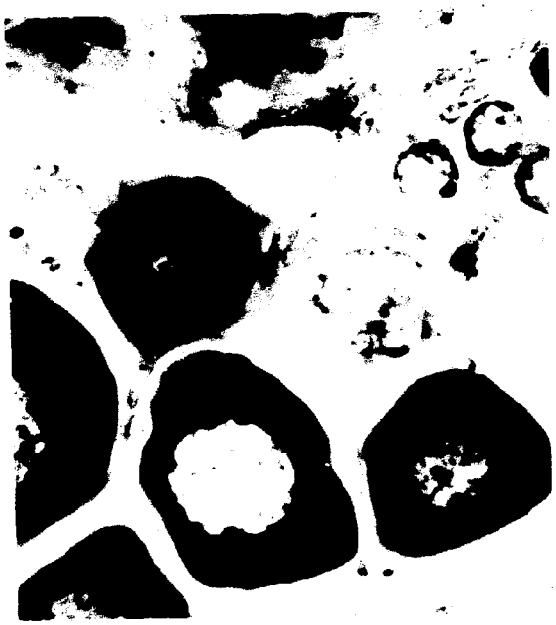

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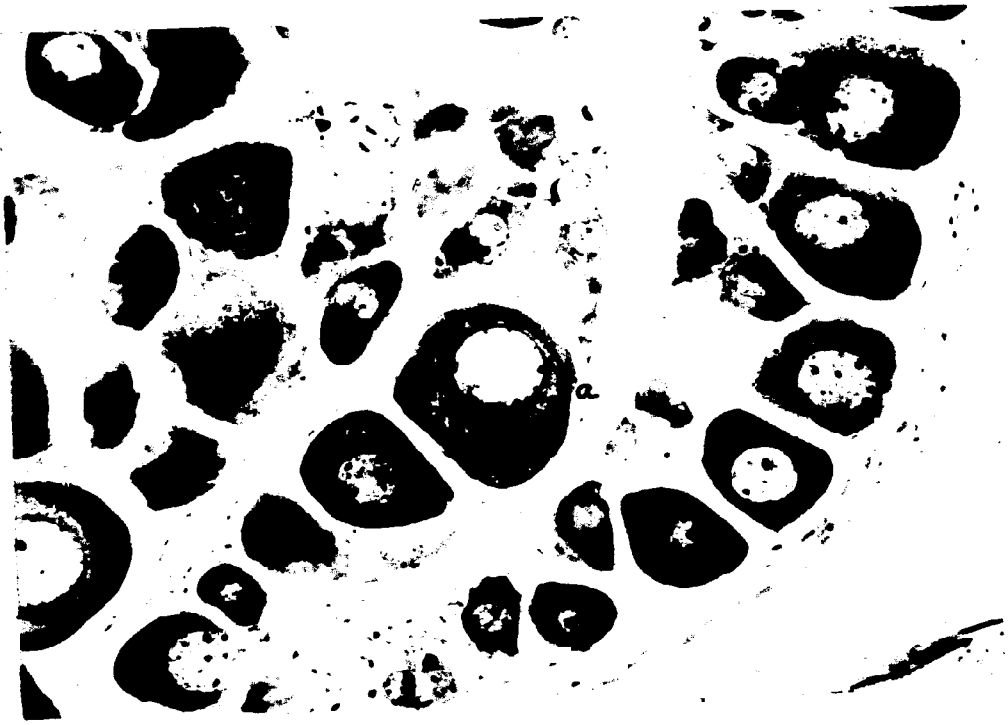

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Plate 21

Explanation of Figures
Figures are unretouched photomicrographs

65 A section through the anterior portion of the testis of a 4.0-centimeter fingerling. Note the delicate mesentery and the thin epithelial covering of the testis. Approximately 350 X.

66 A transverse section of the posterior portion of the testis of a 4.0-centimeter fingerling. Note the higher degree of organization posteriorly than anteriorly. Note that the testocoel (tc) sends branches (1 and 2) into the stroma between the germ cells. Note also the tendency of the germ cells to arrange themselves around a potential lumen.

67 A transverse section of the testis of an 6.0-centimeter fingerling. Note the definite tubular condition of the testis. Observe that the germ cells are located in the walls of the tubules. Approximately 350 X.

65

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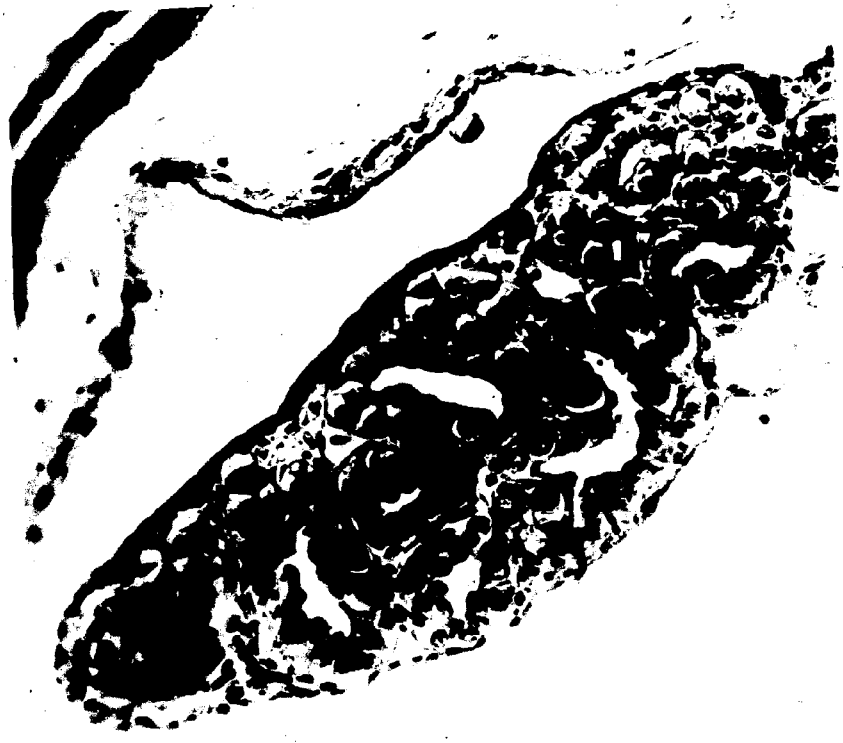

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Plate 22

Explanation of Figures
Figures are unretouched photomicrographs

68 A transverse section of the testis of a 6.0-centimeter fingerling. This section is posterior to that shown in figure 67. The testicular duct (td) lies dorsomedially in the testis and in this section a branch is seen joining it. Approximately 350 X.

69 An enlargement of a portion of figure 67 to show that the tubules are lined with epithelial cells and that the germ cells lie behind them. This is particularly well demonstrated in tubule (a). Approximately 1050 X.

70 An enlargement of a portion of figure 67 to show how the germ cells can lie in the walls of the tubules but still not participate in the formation of its lining membrane. This is shown very clearly in the lower tubule (a) by germ cells (x) and (y). Careful observation will disclose that although germ cell (x) appears to be a part of the surface membrane of the tubule, it is actually underlaid by epithelial cells. Approximately 1050 X.

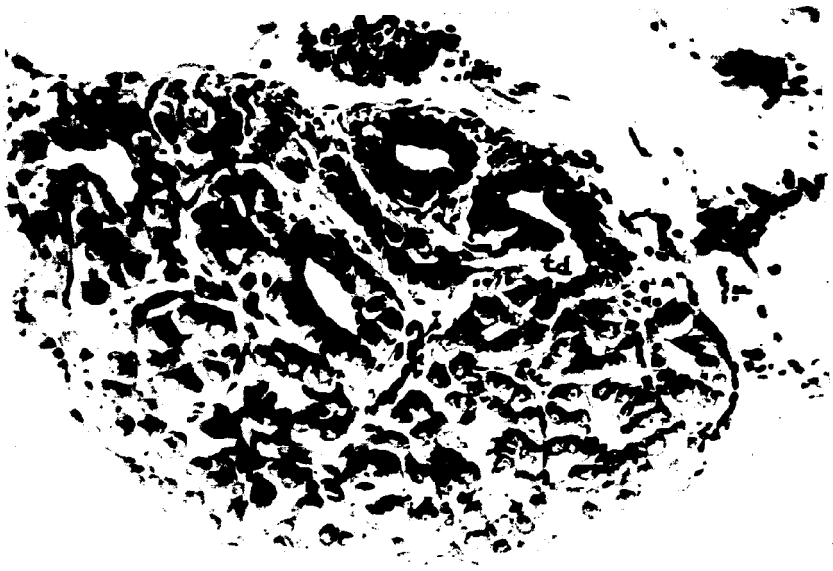

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Plate 23

Explanation of Figures
Figures are unretouched photographs

71 Ventral view of the ovaries and testes of 4.5-centimeter fingerlings. Observe that the ovaries appear as distended elongate bodies while the testes appear as thin elongate strands. Approximately 2.5 X.

72 Ventral view of the ovaries of an 8.0-centimeter bass in situ. Approximately 3 X.

71

72

Plate 24

Explanation of Figures
Figures are unretouched photographs

73 Side view of the testes, in situ, in a 7.0-centimeter bass. The anterioposterior gradation in development is now discernable, grossly. The urinary bladder may be seen near the left hand margin of the figure. Approximately 4 X.

73