OVARIAN MORPHOLOGY AND EARLY EMBRYOLOGY OF THE PEDICULATE FISHES *ANTENNARIUS* AND *HISTRIO*

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BULLETIN
OF THE
AMERICAN MUSEUM OF NATURAL HISTORY
VOLUME 114 : ARTICLE 4 NEW YORK : 1958

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BULLETIN OF THE AMERICAN MUSEUM OF NATURAL HISTORY
Volume 114, article 4, pages 327-372, text figures 1, 2,
plates 47–74, tables 1, 2

Issued March 17, 1958

Price: $1.50 a copy

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INTRODUCTION

THE HIGHLY SPECIALIZED pediculate fishes, entirely marine, have usually been considered as not suitable for laboratory experimentation of any extensive kind. What little is known of the manner of life of these fishes has been mostly worked out in northern field stations on *Lophius*. This is one of the larger pediculates and is not easily handled in aquaria nor is it well suited to the shallow waters that such containers are necessarily limited to. Although *Antennarius*, *Histrio*, and *Ogcocephalus* have been generally accessible to southern field stations, there have been few attempts to study the physiology, reproduction, or other similar matters until quite recently. Not only is it possible to reduce these creatures to the status of laboratory animals in these southern laboratories, it is now also possible to maintain them satisfactorily in city laboratories with fairly simple apparatus, as much of the work of the present report attests. The availability of running sea water in the Lerner Marine Laboratory at Bimini and the establishment of a circulating seawater system in the laboratories of the Department of Fishes of the American Museum of Natural History in New York created an opportunity to make observations on these species which demonstrated their value as experimental material.

The eggs of *Lophius*, *Antennarius*, and *Histrio* are released embedded in rafts of mucoid, gelatinous material. It would appear from all physical evidence that *Ogcocephalus* also releases a similar raft, although such a raft has not yet been discovered or identified. The egg raft of *Lophius* was described as early as 1882 by Agassiz who estimated its size as 2 to 3 feet wide and 25 to 30 feet long. These rafts are fairly familiar to fisherman of the northeast coast of the United States where they are known as "purple veils." They are also well known off the Scottish and Cornish coasts. The rafts released by *Antennarius* and *Histrio*, while relatively enormous compared to the size of the fish that spawn them, are nowhere near so large as the raft of *Lophius*. *Antennarius* rafts seen in the Bahamas may reach a length of 3 feet and a width of 5 or 6 inches. The *Histrio* raft is still smaller and, depending on the size of the fish, may range from 3 to 12 inches in length by 1 to 2 inches in width. The rafts of all known species contain thousands of ova. The eggs offer good experimental material, as the yolk is clear and devoid of granules or oil droplets which obscure vision of development in many forms. For the same reason, the relatively late development of melanophores and their absence in the yolk sac make these embryos useful to the investigator in embryology.

The production of these massive gelatinous egg rafts, which are scrolled on either end in a characteristic fashion, prompted curiosity as to the anatomy and histology of the ovary, and the mechanics involved in the release of the raft from an organ only a fraction of its size. The present report traces the anatomy and histology of the ovaries of *Histrio* and *Antennarius*, with a few notes on the ovarian anatomy of a single female batfish, *Ogcocephalus*, which was available at the same time. The early embryology of the first two species is described, that of *Antennarius* in greater detail than that of *Histrio*. The ova from both species were obtained from fish kept in the laboratory tanks, where normal spawning behavior occurred. Teleost embryology that includes histogenesis and organogenesis is known mainly from freshwater forms and those marine or brackishwater species that lay demersal eggs. The demersal eggs of some species, as *Fundulus*, are more readily available to the investigator because of the ease with which the adults can be collected and induced to spawn in captivity. Other popular experimental material included species the larvae of which are not hatched until they are well along in development and more or less ready to shift for themselves in the proper environment. These often belong to the species that demonstrate some form of parental care or brooding behavior as part of their reproductive pattern. Such behavior helps to bridge the gap between the times when the fry are completely helpless and when they are relatively able to take care of themselves. The problem of feeding such larvae in captivity is not insurmountable, for they are usually big enough to take infusoria

or brine shrimp as soon as the yolk sac has been absorbed. The embryological development of these forms in general follows the classical vertebrate pattern characterized by early establishment of vitelline circulation for the absorption of food material from the yolk and the early production of hemoglobin-containing blood cells for the transportation of oxygen to rapidly developing tissues.

Aside from studies of gross development, the embryology of marine pelagic larvae is not so well known. The early embryology of several forms, particularly those of commercial importance such as the cod (Meek, 1924), has been described, but little if any experimental work has been performed on them. Not only are the larvae notoriously difficult to raise in captivity, but many of the adults do not take readily to confinement and will not reproduce in tanks.

The critical period in the life history of the larvae appears to be the time at which the yolk is completely absorbed and the larvae must take on feeding habits. It is possible that the feeding habits of many marine forms are so specialized that the food is confined to only a few types of organisms. The problem of the aquarist is to find a food that is acceptable and small enough. In the case of *Histrio*, for instance, newly hatched brine shrimp are useless for feeding as they are as large or larger than the larvae. Other factors that bear on the critical period in the life of pelagic larvae are considered in the discussion.

Thanks are due many people for their help with this report. Dr. C. M. Breder, Jr., made many helpful suggestions throughout the course of the work and critically read the manuscript, as did Dr. Phyllis Cahn. Dr. Louis A. Krumholz collected the *Antennarius* and aided in the time-consuming task of taking photomicrographs and making observations of the developing embryos. Mrs. Marie Lou Campbell cared for the fish in the American Museum laboratories in New York and kept continuous and accurate records of their spawnings and observations on the morphology of the egg rafts. Dr. Vladimir Walters collected the *Histrio* from floating Sargassum weed and made sure that they arrived alive in New York.

This work was supported in part by a grant from the National Science Foundation.

MATERIALS AND METHODS

All the fishes used were collected at the Lerner Marine Laboratory, Bimini, Bahamas. The two *Antennarius* that provided the fertilized egg rafts were collected in late April at about 10 P.M. They were discovered in the beam of a flashlight on the bottom under the laboratory dock, near the base of one of the pilings. The two fishes were not more than 6 inches apart. They were transferred to a 15-gallon tank of running sea water in the laboratory where they spawned the next day. The male was identified as *Antennarius scaber;* he was light tan in color patterned with darker grayish green vermiculations. The skin contained many protuberances or tufts. The female was identified as *Antennarius nuttingi;* she was solid black in color and smooth-skinned. The identifications were made on the basis of the key of Barbour (1942). There is no reason to suppose that these fishes, about to spawn in a state of nature, were crossing specific lines. The question of whether or not these truly represent two species does not come within the province of this report.

The *Histrio* were collected from floating Sargassum weed in December. Several lots of very small fish were shipped to the laboratory in New York where they were maintained in 15-gallon tanks in a circulating seawater system and were fed at first on young *Lebistes*. As they increased in size, they were fed larger fish until they were taking 2-inch *Astyanax* at the time this report was written. A description of the circulating system is given by Breder (1957). Under these conditions the *Histrio* matured and spawned frequently, and the larvae were maintained until their development was slightly more advanced than had previously been described (Mosher, 1954).

The egg raft that provided the *Antennarius* embryos was divided in two; one-half was kept in a 15-gallon tank of running sea water in the laboratory under a skylight; the other half was maintained in a small plankton net fixed to a wooden frame floating in the sea off

the laboratory dock. The only difference noted in the development of the two groups was an earlier appearance of pigmentation in the embryos kept in the plankton net. Melanin granules appeared in the melanophores of this group two or three hours earlier than in those kept in the laboratory. This is undoubtedly a result of the brighter light in the open and perhaps the presence of ultra-violet which was blocked by the glass of the skylight in the laboratory. The temperature of the sea water during the period of embryonic development of the *Antennarius* varied between 21° and 27° C.

Developing *Histrio* larvae were brought to their most advanced stage of development in the New York laboratory in a tank of standing sea water provided with illumination from a 100-watt bulb placed on a glass plate immediately over the tank 12 hours a day. Other diffuse illumination was provided by fluorescent light bulbs of the "warm white" variety. No daylight was ever used, and the lighting was automatically regulated; 12 hours of light alternated with 12 hours of darkness. The temperature in this tank was variable, dropping at night to normal room temperature and rising under the light as high as 30° C.

The developing eggs and larvae were fixed in Bouin's picro-acetic-formol. Before the period of hatching, pieces of the raft were cut off and dropped in the fixing fluid. The gelatinous raft proved to be almost impervious to the entrance of the fixative, so that, as a consequence, only those ova next to the cut were properly fixed. For proper fixation, the developing ova should be removed from the raft.

Whole mounts were stained in either alizarin red S or hematoxylin. Alizarin imparted a pale reddish tinge to the whole embryo and was more useful than hematoxylin for study of the pigment cells. Hematoxylin demonstrated other developing structures more clearly. The Bouin-fixed larvae were washed for about half an hour in 35 per cent alcohol. They were stained in dilute Harris' hematoxylin. A few drops of the standard hematoxylin solution were added to 35 per cent alcohol, the stain being diluted enough so that the larvae were visible in the solution and the staining could be controlled under the dissecting microscope. When sufficiently stained, the larvae were transferred to fresh 35 per cent alcohol, then for a few minutes each to 70 per cent, 95 per cent, absolute alcohol, and xylol. They were mounted in Permount. The procedure for alizarin staining was the same except that the stain was dissolved in 70 per cent alcohol. If the embryos were promptly carried through the technique and not allowed to remain in either the fixative or the alcohols too long, shrinkage was minimum. Two special stains were used on whole mounts: toluidine blue for demonstration of mucin and Herxheimer's scarlet red for fat. In both these instances the embryos were mounted in glycerin.

The embryos to be sectioned were submitted to a modification of Rugh's technique (1948). After Bouin fixation they were washed in 70 per cent alochol and then transferred to dioxan. They were next placed in the paraffin oven at 60° C. in a covered container, in a mixture of five parts dioxan to one part of xylol, to which paraffin had been added. From this mixture they were transferred to pure paraffin. Each step required only about half an hour. This same embedding method was also useful for the large ovaries when longer infiltration times were employed. The yolk remained soft and did not give the sectioning difficulties that are commonly experienced when usual embedding methods are employed.

The ovaries were sectioned of six *Histrio* (two immature females, one adult during the process of ovulation, one a few hours after spawning, one six days after spawning, and one 13 days after spawning). One *Antennarius* ovary and one *Ogcocephalus* ovary were also sectioned.

Photomicrographs of developing embryos and larvae were taken with the compound microscope, the material to be photographed being immersed in sea water. *Antennarius* pictures were taken at a magnification of ×115. *Histrio* photographs were taken at a magnification of ×85. The two can readily be distinguished in the plates by their different relative sizes. In life, the *Histrio* eggs are slightly smaller than those of *Antennarius*. Fertilized *Histrio* ova measured about 0.6 mm. by 0.5 mm., and fertilized *Antennarius* ova measured about 0.65 mm. by 0.7 mm.

MORPHOLOGY

HISTRIO OVARY

THE *Histrio* OVARY consists of two glands which are fused in the midline over the region where the short oviduct is found. It thus forms a single organ, as there is no septum present dividing the two halves. The ovary is a flattened, ribbon-shaped sac, the long axis of which lies transversely in the abdominal cavity at nearly right angles to the antero-posterior axis of the fish. The organ is in the shape of a double scroll; the ribbon is equally rolled in from each distal tip towards the midline. The direction of the roll is dorsal to ventral. These features can be seen in plate 47, figures 1 and 2. Because the ribbon of the ovary is thicker in the center and tapers towards each lateral edge, it is provided with a double curvature which makes it impossible to unroll as completely as if it were a simple flat strip. This can be seen in plate 47, figure 3, in the unrolled side of the ovary on the left. In plate 47, figure 1, the photograph shows a ventral view of the dissected ovary from a female 13 days after spawning. Figure 2 of the same plate is a dorsal view of the same ovary, showing the fusion of the two sides of the organ in the middorsal line. Figure 3 is a ventral view of the ovary from a fish which had spawned only a few hours previously. These organs were fixed *in situ;* that is, the whole fish was placed in the fixing solution, and the abdominal cavity was opened to allow penetration of the fluid, but the ovaries were not removed until fixation was complete. The unrolled condition of the right ovary in figure 3 was not caused by any manipulation but represents the condition as it was in the body. It seems possible that this condition could occur on both sides of the organ at the time of spawning, thus facilitating the release of the large egg raft.

The *Histro* ovary is morphologically developed at a very early age, when the fish are only about 15 mm. in total length. Plate 48, figure 1, is a photomicrograph of a section through the fused region in the immature ovary and shows the double scroll characteristic of both the ovary and the released egg raft in this species. The lumen is not centrally located but is at one side. The ovigerous tissue extends into the lumen in the form of lamellae from one wall only, the inner wall of the scroll. The invaginations of connective tissue from the wall of the ovary bearing ovigerous tissue and extending into the ovarian lumen are called lamellae in this report, in accordance with customary usage. However, these invaginations are not flat or leaf-like; rather the stalk is cylindrical at the base, and the subdivisions branching into the lumen are in the nature of tufts. The pores in the egg raft, which are described below, are therefore round. If the ovigerous invaginations were true lamellae, the pore would be elliptical in shape. In the immature organ, the extreme thinness of the non-ovigerous wall is seen in the lower left of the photograph where it has been pulled away from the inner wall. The lumen is thus bounded by ovigerous tissue on one side and the connective tissue stroma on the other (pl. 48, fig. 2). At this early stage the stroma which forms the ovarian wall is extremely thin. On the side bearing the ovigerous tissue it consists of a few strands of collagenous tissue covered by the single-celled mesothelium. A few fibers enter the lamellae, and an occasional capillary is seen in the wall. The ovigerous lamellae are covered by a single-celled layer of epithelium. The opposite wall which is without ovigerous tissue consists of only two layers of cells, one being the outer mesothelial layer and the other an inner epithelial layer. The lining of the short oviduct is composed of a single layer of epithelial cells which is continous with the epithelial layer lining the lumen of the ovary. This is seen in longitudinal section in plate 48, figure 3.

Figure 4 of the same plate is a photomicrograph taken through a transverse section of a formalin-fixed mature ovary 13 days after spawning, showing the arrangement of ova and follicles in the lamellae. Ova of various stages are seen, the most mature ones undoubtedly being those that would have been ovulated at the next spawning time. The stroma of the capsule has increased greatly in

volume and consists of both collagenous tissue and smooth muscle. The epithelium covering the lamellae and lining the opposite side of the lumen shows different characteristics, being more or less squamous where it covers the follicles, and high columnar or cuboidal where it covers the inside of the connective-tissue capsule. The high columnar cells frequently display extruded material on the lumen side of the cell; the cuboidal cells are for the most part free of these processes. Plate 48, figure 1, shows a portion of the stroma where two sides of the ovarian wall meet in the coiled part of the ovary. The ovarian lumen is seen both in the upper right and lower left parts of the photograph. The cuboidal nature of the lining is seen on the lower left; the high columnar cells are on the upper right. It is apparent that these epithelial cells become altered during the cyclical activity of the ovary and that they secrete the gelatinous envelope which surrounds the mass of ovulated eggs and gives the extruded raft its characteristic shape. A few ova in the process of being resorbed are noted in this ovary, but it contains mainly small ovocytes of various sizes and large ripening ova filled with heavy granular yolk.

Plate 49, figure 2, shows the arrangement of cell layers about the mature ovum. The most distal layer consists of the epithelium covering the lamellae, cuboidal in this instance and bearing extrusion products. The next cellular layer consists of a very thin, endothelial-like covering, the long attenuated nuclei of which can be seen in the photograph. This layer contains capillaries and covers the follicle cells which surround the egg. Immediately beneath the follicular layer is the chorionic membrane surrounding the yolk, the clear, non-cellular band in the photograph. At ovulation, follicular, endothelial, and lamellar epithelial layers are ruptured to set the ova free in the lumen. These histological details can also be seen in some of the following figures in which Bouin-fixation gave a more normal appearance to the tissue.

Study of a *Histrio* ovary six days after spawning (pl. 49, fig. 3) reveals a complete absence of ripe ova. After ovulation the lamellar epithelium and the endothelial layer are reconstituted so that the lamellae are repaired and intact. The follicle cells multiply and fill in the collapsed empty follicle which is bounded at this time by the thin endothelial wall. The proliferation of follicle cells takes place also in cases in which the eggs have not been ovulated. The follicular cells surrounding these resorbing ova appear to have some phagocytic function, as their cytoplasm is more or less filled with ingested yolk particles. Certain stages of this process resemble the so-called "corpora lutea" described by Bretschneider and De Wit (1947) for *Rhodeus* (pl. 49, fig. 4).

Vascularization of the entire organ is tremendously increased at this period. Plate 50, figure 1, shows the increase in capillaries and sinuses within the lamellae. Engorged blood vessels are also obvious in the ovarian wall. In as much as this intense vascularization is correlated with the stage of regression of the ovary, it seems logical to assume that it is concerned with the clearing of the organ of debris and unovulated eggs before the next group of eggs to be spawned has begun to accumulate yolk.

The epithelial lining of the lumen at this time consists almost entirely of cuboidal or high cuboidal cells. When stained with Masson's trichrome stain, the chromatin of the nuclei is stained with hematoxylin, and the remainder of the nuclei and the proximal cytoplasm are stained red with ponceau. The distal edges of the cells are stained lightly with Fast Green, and similarly stained, very fine protrusions extend irregularly into the lumen. Bands of smooth muscle which are seen in the connective-tissue wall are stained light red with ponceau and are easily differentiated from the green-staining collagen.

Sections of the ovary pictured in plate 47, figure 3, fixed soon after spawning, show many unreleased ova (pl. 50, fig. 2). Many of these were obviously left over from previous spawnings, in as much as two types of resorbing eggs are readily distinguished, one in a more advanced stage of resorbtion than the other. In plate 50, figure 3, the follicle on the left is one remaining from the most recent spawning; follicle cells are large with vesicular nuclei. The follicle on the right is older and in a more advanced state of resportion. Vesicular nuclei are still present, but cell outlines have nearly disappeared, and there is much cellular debris, including pycnotic nuclei. Part of a ripening ovum is seen within the same lamella. The tips of some of the lamellae are

filled with acellular material resembling coagulated serum.

This particular fish was a large individual of unknown age which had been in captivity for many weeks. The females of both *Antennarius* and *Histrio* consistently lay their eggs in the absence of a male. The absence of a male at the time the egg rafts were released may have contributed to incomplete ovulations. In other species, the presence of the male and performance of the courtship pattern are required for ovulation to take place. The release of the egg raft occurred some time during the night, and the ovary was fixed early the following morning. Not more than 12 hours could have elapsed between release of the raft and fixation, yet a tremendous increase in follicle cell proliferation had already taken place.

The epithelial lining of the lumen is pulled away from the connective tissue wall in many places, probably an artifact resulting from the fixation process. The multiple folds of this lining seen in plate 50, figure 4, probably result from the shrinkage of the ovarian wall after release of the egg raft which had distended the wall so greatly not long before.

According to Cunningham (1898) the most essential characteristic of the spent ovary in *Pleuronectes* is the presence of collapsed follicles from which the eggs have been ruptured. However, he admits that these are very difficult to distinguish in section and that their repair is very rapid. The egg escapes from the follicle, leaving the wall of the follicle open to the surface of the lamella, but this opening soon closes, and the cavity disappears as a result of the contraction of the walls. The cavity is also obliterated by the proliferation of cells derived from the follicular epithelium. The same author describes resorbing eggs, ova that have died before their maturation was complete. He observed these to be very common in immature ovaries of plaice. These dead ova are cleared up by a proliferation of cells from the wall of the follicle. Cunningham was not sure where these cells came from but believed that they were derived from the connective-tissue cells of the stroma, rather than from the follicle cells.

The structure of the walls of the ovary is best seen in the ovary that is in a resting state (pl. 51, fig. 1). The presence of large ova distends the walls so that the various components are difficult to distinguish. In the spent ovary, the walls are thicker and are seen to be composed of collagenous tissue and smooth muscle. The outside wall is thinner than the inside wall which bears the lamellae. A band of collagenous tissue containing many small blood vessels lies immediately beneath the lamellar epithelium in the outside wall. A wider band of heavier collagenous tissue forms the most distal part of the wall, and between these two collagenous layers lies a wide band of smooth muscle. Near the epithelial side the muscle fibers run longitudinally in the section, that is, in the direction of the long axis of the unrolled organ. Muscle fibers between these and the outside collagenous band are seen either in oblique or transverse section. The inside wall of the scrolled ovary shows the same band of collagenous tissue containing small blood vessels next to the epithelial lining. A wide band of longitudinal and oblique muscle fibers joins this. Smooth muscle and collagenous fibers are carried up into the lamellae also, where they are seen to terminate immediately beneath the lamellar epithelium. Both types of fibers run lengthwise of the lamellae. Contraction of these muscle fibers should serve to shorten the lamellae. Next to the band of smooth muscle in the inside ovarian wall is a layer containing rather large blood vessels, both arteries and veins, and nerves. Distal to this layer is another band of smooth muscle, the fibers of which are seen mainly in transverse section. This layer in turn is covered by the peripheral collagenous tissue band.

The ovarian wall is therefore provided with musculature which under proper stimulation could produce a peristaltic-like wave and help push out the egg raft at the time of its release, or loosen the mucoid, gelatinous lining which covers the egg mass after ovulation. In spite of the recent release of the raft by this particular female, sections of this ovary stained with toluidine blue fail to show any evidence of metachromatic staining in any elements, not even among the cells of the folded lining.

During late courtship the female becomes extremely swollen with eggs, and the supposition is that ovulation occurs at this time. The blood vessels of the ovary become engorged

with blood, and the skin of the abdomen is stretched so tightly that the largest ovarian vessels are visible from the outside of the fish. The posterior edge of the ovarian scroll is thus outlined by two large blood vessels that run along the edge of the scroll. With a 5-power lens, the eggs can be seen also through the outside of the fish in unpigmented areas. These appear as small, opaque, white spots and probably represent the ova that will remain in the ovary, in as much as the ovulated eggs are clear.

The tightness of the scroll is necessarily loosened by the increased size of the ovarian lumen which is filled with eggs and the mucoid material of the raft.

From a female anesthetized and dissected during the process of ovulation, it was seen that ovulation first occurs at the innermost part of the scroll and proceeds outwardly. In other words, if the scroll were unrolled, the beginning ovulation would be seen at the extreme lateral tips and would progress towards the fused portion. In the dissected female, one side of the ovary showed a larger area of ovulated eggs than the other, although the process had started in both sides. When the ovarian capsule over the ovulated portion was split, a portion of the raft could be pulled free. It is to be noted that these ova were already encased in the gelatinous envelope. They did not separate and come out singly. A portion of the gelatinous envelope could be pulled out of the vent, but this contained no ova.

Another female *Histrio* was killed during the process of ovulation. The fish was anesthetized with 1 per cent urethane before being placed in Bouin's fluid. After fixation, the ovary was dissected out and sectioned. This particular female had been raised in the laboratory from a very small size, and thus it was known that this would have been her first spawning. This ovary, therefore, contained no resorbing eggs left over from previous spawnings. However, an occasional ovum in the process of being resorbed was seen, representing an egg the proper development of which had been inhibited in some way. Similar resorbing ova were described by Cunningham (1898) for immature ovaries of *Pleuronectes*.

The lumen of the ovary was filled with very large eggs. These were colored with eosin when sections were stained with hematoxylin and esoin, or with Fast Green when Masson's trichrome stain was used. Only the rim of the egg was stained orthochromatically with dilute toluidine blue staining; the mass of the yolk remained unstained in the ripe ova. The yolk was clear and homogeneous. Unripe ova showed a granular or particulate cytoplasm, much of which was stained with hematoxylin, ponceau, or toluidine blue, depending upon which staining technique was used.

The sections were greatly distorted during the fixing and embedding processes, some elements showed sthrinkage and some swelling. The eggs had shrunk so that their outlines were no longer rounded and smooth, while the bands of mucoid material surrounding them had stretched so that unnatural convolutions were seen. The sections are therefore, somewhat difficult to interpret. Plate 51, figure 2, is a photomicorgraph of a section of this ovulating ovary stained with dilute toluidine blue. The large ova are unstained except for orthochromatic staining of their peripheries. The greatly distended wall of the ovary is seen in the upper left of the photograph. This wall also stains orthochromatically as do the immature ova. The convoluted material between the large ova and between the ova and the wall is mucoid, stains metachromatically, and represents the substance that forms the raft. The elements that form the raft can be seen clearly only in sections stained for mucoid material. Raft precursors appear only as an acellular, granular precipitate in sections stained with eosin or Fast Green, and cannot be properly traced to the cells from which it originates.

The large eggs seen in the lumen are not completely ovulated. Each ovum is surrounded by a single cellular layer. When sections are stained with toluidine blue, this layer of cells stains orthochromatically and is identified as the lamellar epithelium. In areas where the attachment of the ova to the stalk of the lamella can be seen, the relationships of the membranes are as follows: the lamellar epithelium and the endothelial layer are still intact, but the follicular cells are not seen. The whole follicle is vastly distended so that these cell layers are very narrow and squamous in character. The chorionic membrane

is easily distinguished by its clear, hyalin, non-cellular appearance. Within the chorionic membrane a thin layer of protoplasm stains orthochromatically, while the yolk material remains colorless.

A slightly more advanced stage of ovulation is marked by the absence of the endothelial layer. At this time the lamellar cells are engaged in rapidly producing the mucoid material which forms the compartmented structure of the raft. In areas where this elaboration of mucus is occurring (pl. 51, fig. 2) the entire lamellar epithelium takes part, not only those cells that cover the ripe ovum, but those that cover the immature ova and lamellar stalks. After fixation, the mucoid material appears fibrous and vesicular, and the fibrous material can readily be traced to the surfaces of the lamellar epithelial cells. There is no evidence of mucus within the cell bodies as there is in the goblet cells of the skin.

At the foot of the lamella, that is, where it joins the ovarian stroma, there is an abrupt change in the character of the epithelium from a cuboidal type of cell covering the lamella to a high columnar type covering the inside of the connective-tissue wall. Both types of cells produce mucoid material, but the lamellar cells elaborate the clear sheets that form the compartments inside the raft, while the high columnar cells elaborate the heavier striated layer that forms the outside envelope of the raft. Plate 51, figure 3, shows a section of the ovarian wall stained with toluidine blue. The darkest band represents the epithelium lining intensely stained orthochromatically, while the striated material above it is mucus which stains metachromatically.

On each side of the base of the lamella there is a fold in the wall of the ovary where the two types of epithelium join, forming a ring around the lamellar base. This can be seen in text figure 1, and the structure of the raft that corresponds to this region can be seen in plate 51, figure 4.

Structure of the Egg Raft

The structure of the raft is formed by a mucoid impression of the internal surfaces of the ovary, that is, of the epithelial surface of the lamellae and of the walls. After the eggs are fully ovulated, the eggs and the mucoid raft are cast free into the ovarian lumen. This phenomenon apparently does not occur until late in the courtship period, perhaps not until five or 10 minutes before the raft is released for fertilization. The action of the smooth muscle components of the ovarian wall and lamellae probably assists in releasing the mucoid material from attachment to the lamellar epithelium. Late in the courtship period, strong contractions are noted in the abdominal wall. These contractions are a sign that the egg raft will be released within a very few minutes.

The structure of the ovary and the corresponding structure of the raft can best be understood by reference to text figures 1 and 2. Text figure 1 is a diagrammatic reconstruction of a section through one lamella of a *Histrio* ovary. The sequence of events has been telescoped for purposes of explanation, and some features have been omitted for purposes of simplification. Thus in the drawing no immature ova are represented. It should also be understood that mucus is not elaborated by the epithelial components until just before or during ovulation when all the follicles are not intact as they are pictured here. Between the outer wall (OW) and the inner wall (IW) is the lumen of the ovary. The inner side of each wall is lined with epithelium (EP) which secretes the mucoid material of the raft (M) at the appropriate period in the ovarian cycle. The epithelium lining the outer wall is unbroken by any ovigerous tissue and thus elaborates an unbroken sheet of mucoid material which forms the outer mucoid boundary of the extruded raft. The epithelium lining the inner wall also secretes mucoid material which forms the inner boundary of the extruded raft. This epithelial lining is, however, broken by the position of the lamellae, the connective-tissue stroma of which is continuous with that of the inner ovarian wall. At the point where each lamella rises from the wall there is a break in the continuity of the epithelial lining of that wall. At these points the lining epithelium connects with the epithelium covering the lamellae which also secretes mucus at the appropriate time. Mucus from the lining epithelium is striated, while that from the

lamellar epithelium is smooth, and each is easily differentiated in the raft. A pore is formed in the raft on the inner boundary where each lamella appeared in the ovary. The two types of mucus can be seen in plate 51, figure 4. In text figures 1 and 2 the mucoid material is represented by stippling. In text figure 2, which diagrammatically represents the structure of the raft, it can be seen that the form of the raft is determined by the shape of the interior surfaces of the ovary. At ovulation, the ripe ova surrounded by chorionic membranes (Y and C, respectively, in text fig. 1) rupture through three cell layers: the follicle cell layer (F), an endothelial layer (E), and the layer of lamellar epithelium (L). They are still surrounded by the mucoid layer (M). The mucoid layers are cast off with the ripe ova into the lumen, leaving behind the rest of the ovarian elements. Wherever there was solid structure in the ovary, as in the lamellae

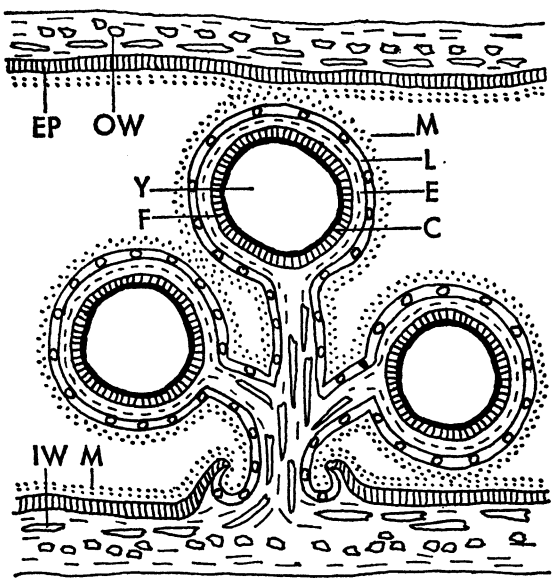

FIG. 1. Diagrammatic representation of a section through *Histrio* ovary, including only one lamella simplified for clarification. Both walls and lamellae contain collagenous and smooth muscle fibers. *Abbreviations:* C, chorionic membrane of the ripe ovum; E, endothelial layer; EP, epithelium lining the ovarian lumen; F, follicular layer; IW, inner wall of the ovary; L, lamellar epithelium; M, mucoid material produced by the lamellar epithelium and the epithelium lining the ovarian lumen; OW, outer wall of the ovary; Y, yolk of the ripe ovum. See text for full explanation.

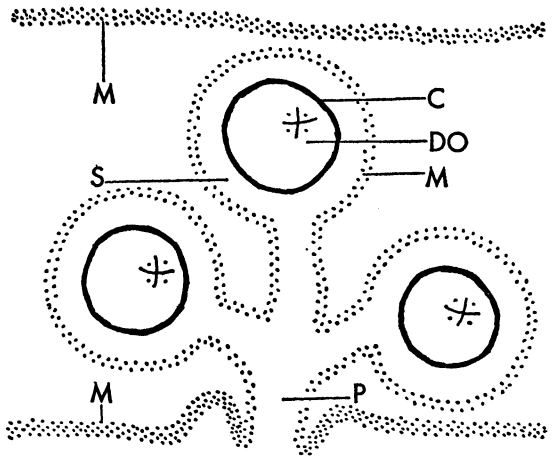

FIG. 2. Diagrammatic representation of the structure of the released egg raft for comparison with figure 1. *Abbreviations:* C, chorionic membrane of the ovum; D, developing ovum; M, mucoid material produced by the lamellar epithelium of the ovary and epithelium lining the ovarian lumen which now forms the mucoid structure of the raft; P, pore open to the outside medium where sea water enters, probably carrying sperm; S, space between the ovulated ovum and mucoid wall filled with sea water in the released raft.

and its banches, there is space in the raft which becomes filled with sea water when extruded. The space formerly occupied by stroma of the lamellae now forms a system of canals and compartments all accessible to the surrounding medium through the pores. This can be seen in text figure 2, in which P represents the pore and DO the developing ovum surrounded by the chorionic membrane C. It is probable that at spawning the sperm reach the ova along with the sea water as the raft is filled with water. Each ovum is thus in a separate compartment surrounded by sea water.

In a freshly extruded raft, occasionally a few compartments are seen which hold two ova, caused by the ripening of both in such close proximity in the ovary that they were covered by a single sheet of lamellar epithelium. This phenomenon is possibly connected with the age of the fish and the number of previous raft releases. As these females aged in the laboratory, it was noted that the production of two ova within one compartment increased in frequency, and the appearance

of three ova in a single compartment was also observed. The ova can be seen to move inside their compartments when pressure is put on the raft at various points. Ciliates and small crustaceans also enter the raft through the pores and can be seen in the compartments with the eggs, some of the crustaceans being large enough to rotate the eggs as they move in the space between the egg and the mucoid wall of the compartment. Although confined within the raft, the eggs are free to rotate the lighter portion uppermost, no matter what wave action the raft may encounter. The mucoid, gelatinous structure remains intact for approximately three days, until the time of hatching, when it begins to disintegrate rapidly.

ANTENNARIUS OVARY

One Bouin-fixed *Antennarius* ovary was available for study. This was dissected from an animal that had been kept in captivity in a 15-gallon tank of running sea water for at least a month. During the period of captivity, no egg rafts were released. From the condition of the ovary, studied histologically, it seems probable that a whole generation of ova was not released but was resorbed. The sections show that the tips of all the lamellae contain large ova all in about the same stage of resorption (pl. 52, fig. 1). In this photograph the two darker ova with coarse yolk granules are also in the process of resorption. The process has not been functioning so long in these eggs, which represent ova that have started to be resorbed before reaching maturity. Eggs in this period of resorption are not nearly so numerous as the more advanced type.

The histology of the *Antennarius* ovary is very similar to that of *Histrio*. As a matter of fact, it is doubtful whether they can really be distinguished histologically. In *Antennarius* the lamellae appear to be more blunt and more or less square on their distal surfaces, and the epithelium lining the lumen appears more highly columnar and the walls somewhat thinner than in *Histrio*, but all these features may well be only a reflection of a certain period in the ovarian cycle. In this particular specimen, the quantity of smooth muscle in the walls is greater in proportion to the amount of collagenous tissue. The epithelial lining of the lumen is very high columnar. When Masson's trichrome stain is used, these cells show a faint staining with Fast Green at their distal tips. When stained with Sokol's modification of Halmi's paraldehyde-fuchsin technique, the distal tips of the cells take the purplish color of paraldehyde-fuschsin. The proximal parts of the cells also stain purplish. Nuclei are unstained and centrally located (pl. 52, fig. 2). Small irregular processes extend into the lumen. The basement membrane is deeply stained with paraldehyde-fuchsin. Lamellar epithelium is low cuboidal and stains similarly in the proximal parts of the cells.

Among the collagenous fibers in the stroma, both within the walls and within the lamellae, are fibers that stain strongly with paraldehyde-fuchsin. These are prominent against the collagenous background which stains with Light Green. The fibers are single for the most part, although occasionally two or three are seen together. The single fibers branch. Sometimes they run in the same general direction as surrounding collagenous fibers; frequently they run at right angles. These are possibly elastic fibers. Similar staining is seen in elastic fibers in the walls of the small arteries in the ovarian stroma. When stained with toluidine blue, these sections stained orthochromatically throughout except for the goblet cells lining a portion of the gut which was accidentally included in the tissue when it was fixed. Mucus within the goblet cells stained metachromatically, a deep purple.

At the base of the lamellae an abrupt change in the character of the epithelium is noted, similar to that in *Histrio*, in which the epithelium lining the lumen meets that covering the lamella. A similar short evagination of the ovarian wall at this point is also present, so that the *Antennarius* egg raft is also provided with pores on the inside surface of the scrolled raft. A surface view of one of these pores is presented in plate 53, figure 1.

In the sectioned ovary, there are rare occurrences of coagulated, acellular material. These are chiefly stained with Fast Green or Light Green, depending on the technique used, but some contain larger, irregular-sized

particles positively stained with paraldehyde-fuchsin. In one of these areas a degenerating egg was found. In toluidine blue-stained sections some of the small particles were stained metachromatically. This is the only possible evidence of any secretion into the ovarian lumen aside from the mucus produced at ovulation. It seems more reasonable to conclude that this is material left behind when the last egg raft was extruded.

When the gross morphology of the ovaries of the two species is considered, the ovary of *Antennarius* shows a longer straight section on either side of the point of fusion of the two organs, and there are more layers to the scrolled parts. In transverse section, the largest *Histrio* ovary processed showed only three rows of ovarian tissue in the scroll, while the *Antennarius* showed five rows. These differences are reflected in the expelled rafts. The fresh *Antennarius* raft can be distinguished not only by its greater size, but by the longer straight section between the scrolled ends.

OGCOCEPHALUS OVARY

Ogcocephalus, as might be anticipated, shows a rather similar ovarian structure, the details of which are given below. From its ovarian structure, it seems probable that at spawning this species also releases an egg raft. The shape of the ovary which is reflected in the shape of the raft of the other species should aid in identification of such an egg mass when collected.

Plate 47, figure 4, is a photograph of a ventral view of the dissected ovary of *Ogcocephalus vespertilio*. The ovaries are also fused at the caudal ends, forming a single organ. However, contrary to the situation found in *Histrio* and *Antennarius*, the ovary lies in the abdominal cavity, with its long axis parallel to the anteroposterior axis of the fish, and a scroll is formed by the rolling in ventrally of both lateral edges of each organ. In *Histro* and *Antennarius* the ovary is rolled from the distal tips of the organ towards the central fused portion; in *Ogcocephalus* the lateral edges of each ovary are rolled ventrally towards the mid-longitudinal line of each side of the organ.

The batfish from which the ovary was dissected for study appeared to be of adult size, measuring 253 mm. in total length. However, in comparison with the ovaries of the other two species the histological appearance of the organ seemed to indicate immaturity (pl. 52, figs. 3 and 4) because of the absence of ripe or resorbing ova. Study of sections stained with toluidine blue revealed that there were patches of acellular material in the lumen which stained metachromatically and which might be remnants of mucoid material left after a spawning. These patches were free in the lumen and showed no relation to any cellular elements.

There are many similarities between the ovaries of *Ogcocephalus* and those of *Antennarius* and *Histrio*. The lumen of the sac is not median but to one side, the inner wall bearing the ovigerous tissue and the outer wall lined with epithelium only. The ova are arranged in lamellae (pl. 52, fig. 3) and in this gland show a nice gradation in size, the smallest eggs being near the wall, the most advanced farthest out in the lumen. The epithelium lining the outer wall and the inner wall between the lamellae is low but is undoubtedly capable of increasing in height as in the other two species. Both walls of the ovary are composed of collagenous tissue, smooth muscle fibers, and small blood vessels. A remarkably different aspect to the batfish ovary is found in the enormous thickness of the ovarian inner wall (pl. 52, fig. 4). This wide band of tissue is composed mainly of loose, collagenous, fibrous material which encloses very large sinuses. These sinuses contain serum or plasma coagulated by the fixing process and contain only rare blood cells. Readily identified arteries and veins, in contrast, contain many erythrocytes as well as plasma. This thickened wall is also plentifully supplied with large nerves.

If the sinuses should become turgid with whole blood or other fluid, the thickness of the ovarian inner wall would be immensely increased. In the histological sections studied here the wall is mainly collapsed. It seems possible that a turgidity, gradual or sudden, under nervous control might contribute to the release of an egg raft at the time of spawning.

FREQUENCY OF EGG LAYING AND MECHANICS OF EGG-RAFT RELEASE

THE FREQUENCY WITH WHICH the female *Histrio* can produce egg rafts is amazing. It is probable that the rafts are not produced so frequently in the normal ecological habitat, in as much as there is some evidence to show that a number of factors may be involved. These include the amount of food available and the presence or absence of the male.

Table 1 gives the dates of released egg rafts of 12 female *Histrio* kept in the laboratory in the closed, circulating, sea-water system. All these fish were collected from Sargassum weed in the Gulf Stream off the island of Bimini, and therefore their ages are unknown. All were small fish undoubtedly sexually immature at the time of collection. The first four fish were part of a collection which arrived in the New York laboratory in early May of 1956. Measurements of these fish are not available. The last eight fish (nos. 5 through 12) arrived in New York on two dates in December of 1956. These were measured on arrival and on three subsequent dates. The measurements were taken on the live fish and are therefore only approximately correct. These data are given in table 2.

These fish are extremely voracious and were kept in separate compartments in order that they might not eat one another. They were fed five days a week, at first, when they were small, on *Lebistes*, later on *Platypoecilus*, and finally on *Astyanax*. It seems quite possible that in nature feeding is not so uniform or so frequent. Certainly a great quantity of nutriment is necessary to build up the yolk in the ripening ova, especially when successive egg masses are matured so frequently. Mosher (1954) records the release of seven egg rafts from a single female *Histrio* during a period of 19 days. Release of the rafts came at approximately three-day intervals, and a male was present through the fifth spawning when the pair were separated because of his aggressive behavior. Intervals between release of egg rafts by a female *Histrio* described by Breder (1949) varied between three and 12 days in the absence of any other individual of the same species. Plenty of food was available in both these instances, small fishes of other species being kept in the same tanks so that the *Histrio* could feed at will.

The dates in table 1 represent the times in which rafts were found in the tanks. Dates in italics indicate those times at which a male was placed with the female and a fertile egg raft was produced. The tanks were observed every day for five days a week, but over the week-end rafts were often released, and the exact date of their release is not known. The date given for these rafts is that of the Monday on which they were discovered, and these instances are marked with an asterisk in the table. Thus for fish no. 6 no time lapse is indicated between the laying of the first and that of the second raft. This female released a raft on Monday, January 21, and the other raft was found in the tank on the morning of the same day, having been released sometime between Friday afternoon and Monday morning. Table 1, therefore, does not give an accurate indication of the exact intervals between egg-raft releases. It does serve to show how prolific these females can be under laboratory conditions.

There are individual variations among the fishes both as to the quantity of food intake and numbers and frequency of egg-raft releases. For example, fish no. 8 ate very little, and this is reflected in the lack of growth shown in table 2. The intervals between release of rafts by this fish run between six and seven days. This is probably not significant, because no. 6, which was also a small fish, ate whenever food was offered and produced a great number of rafts anywhere from three to seven days apart. Number 12, the fish that reached the largest size, was the last one to start producing rafts.

Individual variation is also indicated by the conditions of the sectioned ovaries. The ovary of the *Histrio* that had spawned six days previously was nowhere near in condition to produce another raft within a short time. In plate 49, figure 3, only one ovum in the photograph could possibly be assumed to be filling with yolk material. On the other hand, the ovary of the female which had spawned only a few hours previously (pl. 50, fig. 2) shows many ripening ova.

Mosher (1954) has already described the spawning behavior of *Histrio* and has testified to the instantaneous character of the delivery

TABLE 1

FREQUENCY OF EGG LAYING AMONG INDIVIDUAL *Histrio*

(Asterisks mark dates of Mondays when rafts that had been released during the previous week-end were found in the tank. Dates when a male was placed with the female and a fertile egg raft was produced are in italics. Observations ceased on fish no. 1 on August 30, 1956; on fishes nos. 6, 7, 10, and 11, on April 25, 1957.)

No. of Egg Raft	Fish Nos. 1	2	3	4	5	6	7	8	9	10	11	12
1	6/4/56	6/7/56	6/11/56	6/21/56	1/18/57	1/21/57*	1/28/57	1/30/57	2/1/57	2/4/57	2/4/57	2/22/57
2	6/7/56	6/11/56	6/18/56	*7/12/56*	*1/23/57*	*1/21/57*	*2/7/57*	2/4/57	2/4/57	2/11/57	3/15/57	3/1/57
3	6/11/56	6/18/56	*7/11/56*	7/16/56	*1/28/57*	*1/24/57*	2/18/57	2/11/57	2/11/57	2/18/57	*3/21/57*	3/12/57
4	*6/14/56*	7/19/56	*7/18/56*	7/23/56	Dead	*1/28/57*	3/1/57	2/18/57	*2/14/57*	2/22/57	3/26/57	3/22/57
5	6/21/56	7/23/56	7/23/56	8/2/56		2/4/57	*3/15/57*	2/25/57	2/18/57	*2/28/57*	4/1/57	3/28/57
6	6/25/56	8/2/56	8/2/56	8/16/56		2/8/57	3/22/57	3/1/57	2/22/57	*3/4/57*	4/8/57	4/2/57
7	*6/27/56*	8/16/56	Dead	8/23/56		*2/14/57*	3/25/57	3/7/57	2/25/57	3/11/57	4/15/57	Dead
8	7/2/56	8/23/56		8/27/56		2/19/57	3/28/57	Dead	*2/28/57*	*3/15/57*	4/20/57	
9	*7/5/56*	8/27/56		8/30/56		2/25/57	4/1/57		3/5/57	*3/20/57*		
10	7/9/56	8/30/56		9/4/56		*2/28/57*	4/8/57		3/11/57*	3/25/57		
11	*7/12/56*	9/4/56		9/13/56		3/6/57	*4/10/57*		3/12/57	3/29/57		
12	7/16/56	9/17/56		9/17/56		3/11/57	4/20/57		*3/15/57*	*4/3/57*		
13	7/20/56	10/3/56		9/24/56		3/15/57	4/25/57		3/19/57	4/8/57		
14	7/23/56	10/8/56		9/28/56		*3/18/57*			3/22/57	4/15/57		
15	7/26/26	10/13/56		10/3/56		*3/22/57*			3/25/57	4/25/57		
16	7/30/56	10/17/56		10/8/56		3/28/57			3/28/57			
17	*8/2/56*	10/22/56		10/13/56		4/1/57*			4/1/57			
18	8/7/56	10/26/56		10/17/56		*4/2/57*			*4/3/57*			
19	8/10/56	Dead		10/22/56		4/8/57			4/8/57*			
20	8/13/56			Dead		*4/10/57*			4/9/57			
21	8/16/56					4/15/57			*4/12/57*			
22	8/20/56					4/20/57			4/15/57			
23	8/23/56								Dead			
24	8/27/56											
25	8/30/56											

TABLE 2

INCREASE IN TOTAL LENGTHS (IN MILLIMETERS) OF FEMALE *Histrio* DURING THREE MONTHS

Histrio No.	Date of Arrival 12/7/56	12/26/56	Later Dates 1/15/57	2/6/57	3/7/57
5	25		52	Dead	
6	18		46	57	65
7		33	47	67	87
8		43	57	65	67
9	15		50	66	83
10		23	41	70	80
11	23		50	66	90
12	18		52	70	96

of the raft. This has been confirmed by observations of many spawnings which have taken place in the tanks in the New York laboratories. Repeated observations in tanks devoid of any plants or other obscuring furnishings has yielded additional material.

When the raft is extruded, the center portion comes out of the genital pore first; this is the part that lies immediately over the short oviduct in the fused portion of the ovary. The center portion is rapidly followed by the portion from one side of the ovary, followed in turn by the portion from the other side of the ovary. On rare occasions delivery is not complete. When this happens, the second side of the raft does not follow out but remains within the body of the female, and it may take several minutes before she can rid herself of it. This was also noticed by Mosher (1954).

The rafts are delivered by the females whether a male is present or not, but the presence of a male evidently shortens the length of time required to ovulate. The following facts were accumulated from observations of female *Histrio* kept under conditions described for the New York laboratories, and it is very possible that they may not apply elsewhere. Females in spawning condition are recognized by a slight swelling over the ovaries, which increases until, by the end of the day, the fish are almost completely spherical. When provided with a male, the rafts were released between 4 and 7 P.M. In the absence of a male the rafts were not seen to be released, but were always found in the tank at 9 A.M. the following morning. As the lights were automatically put out at 7 P.M., it is not known whether the unfertilized rafts were released during darkness or with the coming of light on the following day.

The female that was sacrificed to provide sections of an ovulating ovary was killed at what was thought to be the last possible moment before she released the raft. She was in isolation but had been swelling all day and was so inflated that she was hardly capable of swimming upright at the time she was put in anesthesia. In spite of her condition, the sections showed that none of the ova were completely ovulated but were still surrounded by the lamellar epithelium as described above. The second female that was dissected during the so-called ovulatory period proved to have the raft loosened only in the extreme tips of the ovary.

It seems most probable that the extreme swelling of the female is a result not only of the increase in size of the ripe ova but also of the elaboration of mucus. Two muscular functions are involved in the release of the raft. One of these evidently involves the smooth muscle of the ovarian wall and lamellae, the operation of which cannot be seen from the outside of the fish. However, the direction of the muscle fibers in the lamella, running from the base towards the tip, indicates that on contraction the lamella would be shortened, pulling back from the mucoid secretion and thus facilitating a loosening of the mucoid coat from its position next to the lamellar epithelial cells of its origin. Muscle fibers running from the base towards the epithelial lining of the ovarian wall could help to loosen the mucoid coat elaborated by the lining in a similar fashion, while other

muscle fibers in the wall could participate in a peristaltic-like activity first to loosen the raft and finally to expel it.

Muscular activity of the ovary is not unknown among the teleosts, having been described from another but unrelated form which also passes its eggs in a mass. Gaschott (1928) reported rhythmical contractions in the dissected ovary of *Perca fluviatilis* and was of the opinion that this phenomenon was probably common among the Percidae. He thought the contractions were probably used during spawning. He showed a photomicrograph of the musculature of the ovarian wall and reported that the musculature could be stimulated mechanically, electrically, thermally, and chemically, the last by flooding the freshly dissected ovary with a normal solution of calcium chloride.

The second type of muscular function in *Histrio* concerns the contraction of the abdominal muscles which occurs five to 10 minutes before delivery of the raft. The contractions occur along the longitudinal axis of the fish, making an obvious, fairly sharp, longitudinal indentation slightly dorsal to the midline of the ovarian swelling. The contractions differ in different spawnings, even of the same female. They are usually slight at first, becoming progressively more severe in contraction and duration as the time of raft release approaches. Usually each contraction lasts a few seconds, but there is considerable variation, and one female was observed to have only one contraction which lasted for six minutes. The contractions obviously put lateral pressure on the swollen ovary.

From the photograph of plate 47, figure 3, it is obvious that the ovary can become unrolled within the body cavity. Many of the females before spawning have a very lopsided appearance, which, it is easy to imagine, is caused by the unrolling of half of the ovary, as shown in the photograph. The swelling of the eggs and mucoid material would of necessity widen the lumen of the ovary. Even though the ovarian wall is elastic, some restriction is offered to lateral expansion by the connective-tissue stroma, and these two forces would help to unroll the tight scroll, and the raft would be able to slip out more easily. It is believed that one side of the ovary unrolls first and that the first side of the raft to be released comes from the unrolled side of the ovary, immediately followed by the second side.

In order to have the ova surrounded by the raft, the mucoid coat must be separated from the cells which secrete it and it must be slipped off the epithelial lining. It is possible that the most recently elaborated mucus is more fluid than that of the peripheral portion, making a sliding surface next to the epithelium. An abrupt change in the character of the lamellar secretion from a mucoid one to one of a watery consistency would facilitate removal of the mucoid coat, but there is no actual histological evidence to support this. In the ovary sectioned a few hours after spawning, the tips of the reconstituted lamellae are often seen to contain a clear fluid, but this fluid contains a few erythrocytes, and it is seen to occur *inside* the lamella and not in the ovarian lumen. There is some coagulated material which is not mucoid in character within the lumen of this ovary, but it is also found in other ovaries in different stages of the cycle.

EMBRYOLOGY

ANTENNARIUS

THE TWO TYPES of gelatinous material of which the raft is composed are visible in plate 53, figure 1. The first type is the clear jelly-like strands surrounding each ovum. These are thickened into strong resilient sheets making the walls of each egg compartment. The patterns take their form from the corresponding structure of the ovary. In the lower left of the photograph is a surface view of one of the pores open to the surface. The second type of gelatinous material is seen here as a slight stippling resulting from the striations in the outside envelope elaborated in the ovary by the lining epithelium. The compartments usually assume a polyhedral shape owing to the pressures brought on them from adjacent chambers. In whole mounts both types of jelly take a strong hematoxylin stain, and in section both stain positively for mucus with dilute toluidine blue.

Plate 65, figure 1, is a photomicrograph of a section through the outside envelope in a horizontal plane. Although there is nothing here that can be identified as a cell, the polygonal structure suggests the surface of the epithelium from which it was derived.

STAGE 1

The fertilized ovum (pl. 53, fig. 2) is nearly spherical, light yellowish in color, and perfectly clear. No oil droplets or granules are visible in the yolk, and there is no indication of differentiation into animal and vegetal poles. The egg measures 0.65 mm. by 0.70 mm.

STAGE 2

The two-celled ovum results from the first cleavage (pl. 53, fig. 3). The lateral view (pl. 53, fig. 4) shows the blastomeres only slightly elevated above the surrounding yolk surface.

STAGE 3

The four-celled ovum (pl. 54, figs. 1 and 2) is produced by the second cleavage which appears at right angles to the first. In the second batch of eggs studied, this cleavage started 20 minutes after the spawning with a small opening at the center of the first cleavage. The duration of this stage was approximately 20–25 minutes; the temperature of the water was 27° C.

STAGE 4

The eight-celled ovum results from the third cleavage. Two cleavages appear approximately equidistant from the first, at right angles to the line of the second cleavage (pl. 54, fig. 3). This stage and the succeeding one also lasted 20 to 25 minutes.

STAGE 5

The 16-celled ovum (pl. 54, fig. 4) is produced by the fourth cleavage. Two cleavages appear approximately equidistant from the second, cutting across the first and third cleavage lines at right angles. The central four cells are thus completely walled off laterally. The 12 peripheral cells have at least one side open and contiguous with the surface of the yolk.

STAGE 6

The 32-celled ovum (pl. 55, fig. 1) results from the fifth cleavage, and the stage lasted about an hour, with the temperature at 26.5° C. This photograph was taken with a 32-mm. microtessar lens and shows the developing embryos within the gelatinous envelope, the border of which appears at the right side of the picture.

STAGE 7

The 64-celled ovum, lateral view (pl. 55, fig. 2), still shows the peripheral cells with one side open and contiguous with the yolk. No segmentation cavity is yet visible. The cells form a distinct cap still only slightly elevated above the surface of the surrounding yolk.

STAGE 8

The early blastula stage in section shows the germ disk to be four to five cells deep. A narrow segmentation cavity is present. A single layer of tissue with vesicular nuclei two to three times larger than those in the ger-

minal disk forms the floor of the blastocoele, closely applied to the yolk (pl. 65, fig. 2). This syncytium is the central periblast. No marginal periblast is yet present. In neither the living material nor in section can any zone of junction be identified at this time.

Stage 9

The late blastula or early germ ring stage (pl. 55, figs. 3 and 4) is characterized by a thin area in the central mass of the blastodisc, consisting of two or three layers of cells and a peripheral thick area where the cell layers are eight or nine deep ringing the thin central portion. The segmentation cavity, floored by the periblast, is wider and no marginal periblast tissue is seen. The lateral view (pl. 55, fig. 4) shows the beginning of a zone of junction and the segmentation cavity beneath the cap of cells.

Stage 10

At the early gastrula stage the formation of the embryonic shield can be seen as a widened area of the germ ring forming the posterior part of the developing embryo, and establishing the anterior-posterior axis (pl. 56, fig. 1). Figure 2 of the same plate is a lateral view of this stage, showing the deepened cavity and an enlarged zone of junction.

Stage 11

The chief characteristic of the middle gastrula stage seen in the living embryo is the formation of the neural keel. At this time also, in this species, the marginal periblast is present, slowly expanding over the yolk (pl. 56, figs. 3 and 4; pl. 57, figs. 1 and 2). A narrow perivitelline space is also visible. The caudal end of the neural keel slightly overhangs the rim of the marginal periblast in the area of what will be the dorsal lip of the blastopore. Sections show the periblast to cover a little less than half of the surface of the yolk. In sections of embryos at this stage it is possible to distinguish between the ventral entoderm which appears about two cell layers deep and the much thicker overlying layer which consists of the neural plate and probably the mesoderm and presumptive notochord. These elements are seen in sagittal section in plate 65, figure 3.

Stage 12

The late gastrula stages are marked by further differentiation of the ectoderm into a solid core of cells, with small groups of cells on each side in a position comparable to that of the neural crest. These are especially prominent in the anterior part of the neural keel which is advancing rapidly.

Stage 13

The formation of optic vesicles, characteristic of stage 13, can be seen in plate 57, figure 3. The photograph was taken through the ventral surface of the yolk. The two black lines on each side of the neural keel do not represent pigment but are a result of the lighting arrangement. The overlying ectoderm is not yet separated from the neural keel, and the keel and optic vesicles are still solid structures containing no lumina (pl. 66, fig. 1). Some differentiation of mesoderm has taken place at the sides of the keel, particularly in the trunk region.

Stage 14

This stage is marked by several developmental advances: tubulation, development of Kupfer's vesicle, differentiation of a definitive notochord, and pigment formation. In plate 57, figure 4, and plate 58, figure 1, Kupfer's vesicle is prominent. A section through Kupfer's vesicle is seen in plate 65, figure 4. A lumen is present in the primitive gut throughout nearly its whole length except over the region of Kupfer's vesicle where the floor of the gut is still periblast tissue. A lumen is only just appearing in the neural tube which is now separated from the overlying ectoderm (pl. 65, fig. 5). The optic vesicles have invaginated, and lens formation has been initiated. The tiny lens is visible in the photograph of plate 58, figure 1, and in addition some differentiation of the main divisions of the brain can be seen. The auditory placode is present and in some embryos shows a small lumen.

Cells containing melanin granules first make their appearance at this stage. These are not found in the neural crest but next to the neural tube in a lateral position more towards the ventral aspect of the tube than towards the dorsal. They are not visible in the living embryo at this time but can be seen in sections and whole mounts, particularly those

stained with alizarin. Plate 65, figure 5, shows some melanin granules at the left of the neural tube, between the tube and the developing mesoderm. In the photograph, the structure at the upper left is the posterior end of the auditory placode, and neural crest tissue lies between it and the dorsal part of the neural tube. At this stage, this is the farthest anterior position of the melanin-bearing cells which are confined to the trunk region. The cells are not numerous and appear scattered along each side of the neural tube without regard to any mesodermal structures. Somites have not yet begun to form.

Stage 15

Stage 15 is marked by the appearance of somites. Photographs of the living embryos in this stage are seen in plate 58, figures 2 and 3. In plate 58, figure 2, it can be seen that the germ ring is still advancing down over the yolk surface. In this species, the blastopore is very late in closing. Figures 2 and 3 of plate 58 were photographed through the ventral surface of the yolk, as were many other pictures of this series. The heavier embryo tends to rotate the egg so that the lighter part containing the yolk is apt to be uppermost. The auditory placodes are prominent in these photographs, and sections show that there is a definite lumen in the auditory vesicle (pl. 67, fig. 3). The brain is differentiated into the three primitive divisions of telencephalon, diencephalon, and metencephalon. Olfactory placodes are present. Plate 66, figure 3, shows the invagination of the optic cup and developing lens.

The living embryo now prominently displays two rows of developing melanophores. These cannot be seen very well with transmitted light but are readily visible by reflected light and can even be distinguished with the naked eye. Plate 66, figure 5, is a lateral view of an alizarin-stained whole mount which shows the distribution of the pigment. The pigment-bearing cells now have the appearance of mature dendritic melanophores. They are still confined mainly to the trunk region, although there are a few in the head. Those in the more posterior portion have begun to migrate ventrally around the gut. In the photograph, in this more posterior region a slightly clearer area above the pigment layer is the notochord, so that in this area the melanophores have migrated ventrally beneath that structure. It is a question whether those in the head have migrated from the trunk region or are simply cells which were already in place and have only recently acquired melanin granules. Plate 66, figure 4, shows a section with melanophores between the tube and the posterior end of the optic vesicle, the pigmented cells obviously destined to form a part of the choroid coat. Plate 67, figure 4, is a transverse section just posterior to the auditory vesicle. The thin roof of the myelencephalon is visible in this section. Figure 1 of plate 67 shows the melanophores on each side of the gut in the more posterior region, in horizontal section. These developing melanophores appear destined for either the choroid coat of the eye or the covering of the gut, in as much as none appear at this time in the tail region which is by now well developed and in part is free of the yolk surface. From time to time a few isolated melanophores have been described here in *Antennarius* as being seen over the location of the yolk. These are not on the yolk sac surface but are close to the trunk of the embryo within the body of the embryo proper. Contrary to the situation in many developing teleost embryos, melanophores are not seen on the surface of the yolk at any time in *Antennarius* or *Histrio*.

The notochord at this stage is a well-developed structure showing the typical teleost pattern of transverse membranes and "vacuolations." Plate 67, figure 2, shows the cord in sagittal section, with part of the neural tube above and the gut with associated melanophores below. The anterior end of the notochord lies just beneath the caudal limit of the metencephalon, and the posterior end is carried nearly to the end of the tail bud. In most fish embryos there is a subnotochordal rod of cells which is thought to become differentiated eventually into or to contribute to the dorsal aorta. In *Antennarius* this structure cannot be identified, but there is a supranotochordal rod visible in this same photograph which makes a transient appearance. It is present in this stage, but its future disposition could not be determined from the sections.

Somites in the tail region are solid aggre-

gations of rapidly proliferating cells (pl. 67, fig. 5). In the trunk region, the somites show the beginnings of a slight differentiation, the mesiad portion distinguishable from the distal two-thirds of the structure.

The gut is now differentiated into two parts; the pharyngeal or anterior part is composed of cells that are lower and more closely packed than those of the midgut region. The region of transition from one cell type to another is short and sharply demarcated. At its anterior end, the gut rises dorsally, and ventral to it the heart anlage can be identified in sagittal section. At this stage it is represented only by a small lumen the ventral wall of which is one or two cells thick, lying directly on the surface of the yolk. Its dorsal wall lies against the under surface of the gut, and the lumen is in open communication with the yolk sac sinus, the space between the embryo and the yolk sac. At this stage, the heart begins to beat in the living embryo.

Just posterior to the auditory vesicle the gill slit is beginning to form, from the outside medially, but it is not yet broken through. In this stage, also, large granulated cells with round vesicular nuclei become prominent in the outside ectoderm.

Stage 16

Photographs of the living embryos of this stage are seen in plate 58, figure 4, and plate 59, figures 1 and 2. Figures 3 and 4 of plate 59 represent later periods in the same stage. The different parts of the brain are more fully marked, and the optic lobes are pushing out laterally. The lenses are well differentiated. A layer of pigment is formed in the choroid which seems to consist of scattered melanin granules within the choroid cells. The typical melanophore is not found here. Plentiful melanophores surround the gut at the anterior end, where two wing-like aggregations of pigment cells come out at the sides and approach the developing choroid coat of the eye from the dorsal aspect. In living embryos seen by reflected light this resembles a black Y. The presence of melanophores ends abruptly a short distance anterior to the anus; the entire tail is still free of any pigment. An occasional melanophore is seen near the surface of the yolk, always quite close to the embryo.

In plate 59, figure 1, Kupfer's vesicle appears in its normal place, but in figures 2 and 4 the vesicle appears larger than normal and occupies a much more anterior position under the trunk of the embryo. It is not known whether this is abnormal or not. Some of the sectioned embryos of this stage obviously had a median vesicle which was bounded by the open gut on the dorsal side and the periblast cells on the ventral side in the same manner as the normal Kupfer's vesicle in other embryos. From the photographs, it seems that this vesicle might be the swim-bladder, but sections do not confirm the presence of such an organ. In fact, the swim-bladder anlage did not make its appearance throughout the life of the larvae studied for this report. In as much as the adult fish have swim-bladders, this organ must make its appearance at a later period.

The blastopore is finally closed, and the peculiar aggregations of cells in the outside ectoderm are more prominent than before. These form a pattern over the surface seen clearly in plate 59, figure 2. This pebbly appearance is characteristic of both *Antennarius* and *Histrio* embryos and larvae.

The five main divisions of the brain are clearly established, and some of the cranial nerves are forming (pl. 68, fig. 1). Some of the spinal nerves are beginning to form. This can be seen in plate 68, figure 2, which also shows two anlagen of lateral-line sense organs.

Mesenchymal structures are rapidly developing. In plate 63, figure 1, the limb buds, which will become the pectoral fins, can be seen. These are prominent in the hematoxylin-stained whole mounts but virtually invisible in the living embryo. In the whole mount they appear to be forming in the yolk sac wall disconnected from the embryo, but sections show that there is a connection with the main body of the embryo although it consists only of a tenuous thread made up of a double layer of cells. This can be seen in plate 68, figure 2, in the middle left side of the photograph.

The heart is now readily found in sections, being larger and characterized by two distinct types of cells, cuboidal cells lining the lumen and an outer layer of cells with spindle-shaped nuclei resembling fibroblasts. The tube of the heart is open (pl. 68, fig. 4) at both

ends. There is no sign of developing aorta or any other blood vessel to be found. In the mid-trunk region the notochord appears to rest directly upon the gut. The supranotochordal band of cells is still present in this stage. These cells may eventually participate in the formation of the vertebrae.

The gill slit is now completely broken through (pl. 68, fig. 3), and its ventral borders extend farther laterally than the dorsal borders. In plate 59, figure 2, these are seen as bilaterally symmetrical outpushings at the anterior end of the pictured embryo.

Metamerism is becoming established. In the posterior region of the embryo the cells of the somitic mesoderm have become elongated both in respect to cell body and nuclei and are so aligned that they give the diagnostic appearance of developing musculature. This is most obvious in horizontal section.

The liver anlage has appeared, differentiating from the wall of the gut posterior to the limit of the pharyngeal area (pl. 68, fig. 5). The caudal end of the gut also shows a transition in cellular type, the high slender cells characteristic of the midgut region being abruptly replaced by low cuboidal cells just anterior to the place where the anus will appear.

Stage 17

Living embryos in this stage are represented by the four figures of plate 60. This is the stage just before hatching, and figure 4 shows the embryo with tail already free of the yolk. The tail is lashed back and forth vigorously. At this time the raft is rapidly disintegrating. The volume of yolk is still considerable and is prominently vacuolated around the periphery.

Whole mounts show the melanophores to be very large cells with coarse dendritic processes completely encircling the gut. In the anterior region they appear under the brain and extend outward towards the eyes. The retinal pigment appears quite different, the granules are smaller in size and, while extending over the whole retina, are still sparse enough to give the retina a color much less dense than that surrounding the gut. An occasional melanophore seems to be present on the yolk surface but is actually close to the trunk of the embryo, in the lateral mesoderm.

In the brain the third ventricle is very prominent, with a short, thin roof and floor. Mitosis is actively occurring in the brain cells along the borders of the ventricle (pl. 69, fig. 1). The fourth ventricle is extensive, reaching completely over metencephalon and myelencephalon. Its roof is composed of only a single layer of cells which is still closely applied to the under surface of the outside ectoderm. Considerable flexion has occurred in the head region, so that it is now possible to get transverse sections with eyes on the dorsal aspect and otic capsules and gill slit on the ventral aspect of the same section. Mesenchyme is beginning to fill in between the brain and the most proximal part of the choroid.

Lens fibers are differentiated, but the optic cup shows no separation into various layers as yet. Mitotic figures are abundant in the base of the cup next to the pigmented capsule. The otic capsule now contains a large lumen, and its wall has thinned on the distal side to a single row of cells and thickened on the proximal to a depth of three or four rows.

Vacuolations are extremely evident in the outside ectoderm. These are confined within cells that are associated with others filled with a granular substance. In some cases the latter type appear to be multi-nucleated. At frequent intervals along the trunk region the inside layer of the outside ectoderm is differentiated into anlagen of lateral-line sense organs. In some instances these have considerable dimension (pl. 69, fig. 5).

There is some change in the heart from the last stage. The spindle-shaped cells forming the pericardium are more numerous. A single layer of thin cells has appeared inside the cuboidal cell wall, probably the forerunner of the endocardial lining. The heart tube is still open at both ends, the only possible circulation being fluid pumped through the heart taken from the subdermal space on one side and released to the same space on the other. Any sort of coagulum representing what might be precipitated protein is never seen in this space in section. The subdermal space is always perfectly clear in these early stages.

The disappearance of Kupfer's vesicle is characteristic of this stage of development, and the gut is now completely closed from beginning to end.

The fin bud, although not greatly increased in differentiation from the previous stage, is now oriented so that it stands out from the yolk surface. In spite of this, histologically it

consists of nothing more than a mass of undifferentiated, rapidly dividing cells (pl. 69, fig. 3). Outside ectoderm in the caudal region has become closely associated with the main body laterally and reached outward dorsally and ventrally to form the embryonic fin fold.

The pronephric ducts are present (pl. 69, fig. 2). In transverse section these are seen as two small tubes lying on each side and, dorsal to the gut, frequently enveloped by melanophores.

The liver is a little larger in this stage than in the previous one. In the large majority of the embryos this is an out-pocketing from the ventral surface of the gut, but it is also seen dorsally (as fig. 5 of pl. 68 shows), and some embryos show a lateral outpocketing (pl. 69, fig. 4). The staining reaction of the cells of the liver anlage is rapidly changed, and sometimes even before there is an actual evagination the area where it will occur can be identified by the more deeply stained cytoplasm which has a greater affinity for hematoxylin than that of the surrounding cells.

Stage 18

Plate 61, figures 1, 2, and 3, are photomicrographs of the living embryos in this stage, which occurred 75 to 100 hours after spawning. The magnification had to be slightly reduced in order to get the main portions of the larvae shown in figures 1 and 3 into the field. They are obviously older than the larva represented in figure 2, which is shown by the reduced size of the yolk sac.

Many of the hatched larvae are still confined within the remaining meshes of the disintegrating raft. Probably this would not be the case in nature, where the wave action of the surface of the sea would have broken up the raft and released the larvae before this time.

The only obvious change in pigmentation in this stage is a greatly increased deposition of melanin in the choroid and retina, and a few melanophores can be seen to have migrated between the notochord and the neural tube in the anterior region.

The subdermal space is greatly enlarged from the previous stage—partly a result of the shrinking of the yolk sac but mainly a result of the extensive growth of the marginal fins. Musculature is seen only in the myomeres which are closely associated with the notochord, leaving a wide space between the outside membrane and the main body of the larva (pl. 69, fig. 2; pl. 70, fig. 1).

The whole exterior of the larva is covered with a pattern of vacuolated and granular cells. In the alizarin-stained whole mounts the granular cells are seen to be quite large, and the granules are also large and refractive. Plate 70, figure 2, shows the appearance of these cells in a transverse section of the membrane. In section, the granulation is intensely stained with eosin but is not refractive. The vacuoles are clear. The cells are found in the second layer of the membrane. They are separated from the surrounding medium only by a very fine, extremely flat epithelium of which only a nucleus is seen now and then; the cytoplasmic membrane between nuclei is invisible except where distortion has occurred and the layers of the membrane have been split or torn apart. Plate 70, figure 3, is an oblique section through an *Antennarius* larva of a later stage (stage 23) which shows the arrangement of the cells in the dorsal marginal fin. This does not change appreciably as the larva grows older. Figure 4 of the same plate is a photograph of part of a hematoxylin-stained whole mount of *Histrio* in stage 18. At this time, two similar *Histrio* larvae were fixed and stained, one for demonstration of fat and the other for mucus. There were no positive results in either case. A possible function for these cells is considered in the Discussion.

The brain is beginning to show fiber formation. In transverse sections a distinction is seen in that the cellular portions which occupy most of the brain are located dorsal to the much narrower ventral area of non-cellular, eosin-stained material.

The pectoral fins have pushed out so that a 60-degree angle is formed between the insertion of the fin and the body wall posterior to the insertion. They carry the outside ectoderm with them. They are innervated by one or more of the spinal nerves, but nothing resembling muscle cells can be distinguished. Mesenchyme is filling in between the brain and the outside ectoderm from anterior to the eyes posteriorly to the insertion of the fins. Its heaviest concentration is seen between the eyes and the brain and around the gill slit. The tube of the heart shows a twisted condition which is very plain in hematoxylin-stained whole mounts. At this time the ante-

rior end of the pronephric duct is a small, solid ball of cells. The anus is open (pl. 70, fig. 5).

The living embryos are extremely active at this stage when they are hatching. The tail is lashed back and forth spasmodically which aids in ridding the larva of the egg membrane. The membrane seems to split over the head and the larva pushes itself out.

Stage 19

The living embryos of stage 19 are pictured in plate 62, figures 1 and 2. These represent 112 and 122 hours after spawning, respectively, the older larva being distinguishable by the diminished size of the yolk sac. In figure 1 the otic capsules and the thin roof of the fourth ventricle are obvious as well as the myomeres. In figure 2 the pronephric ducts can be seen joined to a small bladder. The lower end of the gut is expanded here also. This phenomenon was seen rather frequently and may represent an abnormality. The larvae are definitely not feeding at this time. This distension of the lower intestine is seen in the stained whole mounts represented in plates 63 and 64 in both *Antennarius* and *Histrio*.

Plate 63, figure 2, is a photomicrograph of stage 19 of *Antennarius* in an alizarin-stained whole mount. It can be seen that there is little if any change in the pigmentation. The otic capsules are clear in this photograph, as well as the pectoral fins.

The ventral portion of the brain is more fully differentiated into fibers, and the optic chiasma is established (pl. 71, fig. 1). The hypothalamic region is becoming marked off from the overlying telencephalon by a thin lateral extension of the third ventricle. The infundibular region of the midbrain reaches down to the dorsal wall of the pharynx, but nothing resembling Rathke's pocket can be identified. At least five layers can be distinguished in the retina, three cellular and two fibrous in addition to the pigmented layer. The rectus muscles of the eyes are beginning to form. Nasal placodes are present.

At some time between this stage and the previous one, the small clumps of cells in the outside ectoderm previously identified as anlagen of lateral-line sense organs virtually disappear. Only rarely can one of these be identified in section. Under low magnification they appear only as a slight thickening in the outside ectoderm, but high magnification reveals the orientation of the nuclei to be at nearly right angles to that in the rest of the membrane.

The pectoral fin has developed an evagination at its distal limit, an outpocketing of the epithelium which will form the membrane in which the fin rays will differentiate (pl. 71, fig. 4).

The heart has changed only to lengthen so that it pushes out farther in a ventral direction.

The liver is closely associated with the dorsal surface of the yolk sac. The cells of the liver are no longer connected with those of the wall of the gut but are now contained within a separate organ. The gut has a pronounced sigmoid curve. In the curve of the first loop, where the gut turns ventrally, the pancreatic anlage is differentiated from the ventral wall. The cells of this organ are distinguished from those of the liver by their smaller size and coarser, more deeply stained chromatin. The periphery of the yolk material is extremely vacuolated, and the wall of the sac composed of periblast cells has thickened.

The anlage of the gill pouches which has been present heretofore as two solid masses of cells immediately ventral to the gill slit (pl. 68, fig. 3) now shows considerable mitotic activity, and there is a slight lamination suggestive of the appearance of gill clefts. Larvae in a later part of this stage show two gill clefts broken through on either side.

The pronephric ducts are joined in the caudal region posterior to the end of the gut where they form a urinary vesicle or bladder. The wall of the bladder is formed of very squamous cells, quite different from the cuboidal nature of the ducts. The anterior ends of the ducts in older larvae form a loop, so that in transverse section four small aggregations of cells are seen. In this area they cannot be called tubes as there is no lumen.

Stage 20

The living embryo in stage 20 is pictured in plate 62, figure 3. The yolk sac here is considerably reduced, and the subdermal space also occupies a smaller area. The lens and cornea can be seen. A very thin wall can be

seen between the yolk sac and the anus which delimits the peritoneal cavity, separating it from the subdermal space.

The eyes are beginning to rotate, so that they face more directly forward to give the binocular vision which is a characteristic of the adult. The layer that will eventually contain rods and cones is sharply distinct from the other layers of the retina; the cells are cuboidal, equal in size, and remarkably equal in height.

The heart is still open at both ends into the subdermal space. There is still no trace of the aorta or other blood vessels.

Vacuolations of the peripheral yolk material are very plain in alizarin-stained whole mounts, as is the tough yolk-sac membrane. Cells of the liver rest directly upon the yolk (pl. 71, fig. 5). The floor of the pharynx is many layers of cells deep, most of the material of which is probably contributed to the developing gill arches and their derivatives. However, in the region where the pharynx narrows to become the gut, a thin thread of cells comes off the ventrolateral area and forms a membrane (pl. 71, fig. 2). This membrane appears to become the peritoneum. At this stage it may not be completely formed, but is visible in the anterior region and in the posterior region (pl. 62, fig. 3). The membrane is so thin that possibly its continuity is destroyed by the distortions caused by histological techniques. There is no evidence in these embryos of any division or split of the somites into somatopleure and splanchnopleure with the coelom formed by the separation of the two parts. The mouth is not yet open, but its position is marked by an indentation in the outside ectoderm. This is not a stomadeum as there is no infolding of the outside ectoderm. The pancreas is represented in some sections by a single or double row of cells surrounding the outside of the gut wall. On the side of the wall next to the liver, liver and pancreas cells exist side by side. The cytoplasm of the pancreatic cells has an increased affinity for hematoxylin.

Stage 20 is marked by the appearance of cartilage in various structures. The diagnostic features of hyalin cartilage are apparent in the otic capsule. A broad thin plate of cartilage is formed in the center of the pectoral fin. Plate 71, figure 4, shows this fin cartilage in longitudinal section of a late part of the stage. A cartilaginous rod, the forerunner of the parasphenoid bone, is present extending from the anterior portion of the larva between the nasal placodes back to the gill bars where it bifurcates. The cartilaginous framework of the gill bars has also been differentiated. Plate 72, figure 3, shows the cartilage of the parasphenoid bone immediately posterior to the bifurcation, cartilages of the gill bars, and the infundibular region of the brain.

Stage 21

Stage 21 is represented in plate 62, figure 4, in a photograph of the living larva. Although the pigmentation covers the yolk-sac area so that the sac cannot be seen here, it is still present in a very reduced state. The large pectoral fins are capable of very rapid movement and are used extensively by the larva in swimming. This stage occurred in 148 hours after spawning.

Plate 63, figure 3, is a microphotograph of an alizarin-stained whole mount in stage 21. The cartilaginous element of the pectoral fin is plain.

The separation between the third and fourth ventricles in the brain is sharply marked off by the development of two columns of cells, one continous with the roof of the third ventricle and one continous with the roof of the fourth ventricle which rises from the cellular portion of the brain.

The proximal portion of the otic capsule shows a differentiation of the lining to form sensory cells. The wall is considerably thickened by an accumulation of fairly large cells with vesicular nuclei. The auditory nerve enters the capsule from the back in this area. Anteriorly on the distal side of the capsule, an aggregation of cells pushes towards the lumen, a forerunner of the septum that will divide the capsule into two chambers. In late embryos of this stage the septum is completed (pl. 72, fig. 1).

Over the head and heart region the cells of the outside ectoderm appear hypertrophied; both granular and vacuolated cells are increased in size. For the first time, a faint, granular, eosin-stained precipitate is seen in the subdermal space indicative of the presence of the fluid which must occupy this space in the living larva.

A thin pericardium is present. Within this one-celled membrane occurs symmetrically, on each side of the heart, a splitting of the membrane into two layers of cells for a short distance (pl. 72, fig. 1). A granular, eosinophilic substance is found between the two layers. When followed through successive sections these are seen to join the body wall where the pectoral fin articulates. A lumen which represents the dorsal aorta can be seen immediately ventral to the notochord in the trunk region. The lumen is enclosed by fine endothelium, and the vessel is bounded on left and right by the lateral musculature. The aorta is not connected with the heart at this stage. Anterior to the level of the pectoral fin there is no ventral wall to the aortic space, and consequently no lumen is present. Posteriorly, in the region of the caudal peduncle, the aortic lumen is divided into two compartments by a transverse septum.

The mouth is open at this stage (pl. 72, fig. 2). No invagination of the outside ectoderm takes place. Cartilages of the mandibular bones are present.

The liver cells show large vacuolations. What is left of the yolk is no longer homogeneous but is broken up into large flakes. The yolk-sac membrane has widened and takes a stronger hematoxylin stain; the nuclei are hyperchromatic. The vacuolated liver cells rest directly upon the yolk; the yolk-sac membrane is not present between cells and yolk material.

A large lumen has appeared at the side of the pancreas, between the pancreas and the yolk sac but connected to the pancreas. This is possibly a forerunner of the pancreatic duct. The pancreatic cells have assumed the diagnostic histological features of adult pancreatic tissue, with deeply stained basal nuclei and refractive eosinophilic zymogen granules in the cytoplasm.

The heart has two definite chambers. The sinus venosus which is open to the subdermal space is marked off from a more anterior chamber by a constriction in the wall of the tube. There is not yet any sign of a ventral aorta.

Mesenchymal cells are most numerous around the developing cartilages, anteriorly around the parasphenoid and around the jaws and gill arches.

The olfactory nerves are forming, connecting the anterior part of the proencephalon with the olfactory placodes which were differentiated from the outside ectoderm.

Stage 22

Plate 63, figure 4, represents a ventral view, and plate 63, figure 5, represents a lateral view, of *Antennarius* in stage 22. Figure 6 of the same plate is a larva in the late part of the same stage. All are hematoxylin-stained whole mounts. The cartilage of the basal fin bone is clear in figure 4 as well as the increased area of the distal membrane where the fin rays will appear. The lateral views of figures 5 and 6 of plate 63 show the increased rotation of the eyes to face forward, as well as the remaining yolk sac. This stage occurred about 172 hours after fertilization.

Melanophores are found in the transverse septum, in the peritoneum, around the pronephric ducts and gut, and on the ventral surface of the brain. No melanophores are seen in the outside ectoderm. The choroid coat of the eye is heavily invested with melanin, and the pigmented layer of the retina is well established. In the living embryo, guanin can be seen reflecting light from the surface of the eyeballs.

In the brain the most ventral area of the infundibulum has differentiated into a small aggregation of cells probably representing the neurohypophysis anlage. There is no sign of anything resembling Rathke's pocket or of any tissue from the dorsal pharyngeal lining reaching up to come into contact with the infundibulum. Until the time of the death of these embryos, there is no clue to be found in the sections showing where the anlage of the adenohypophysis arises.

Some of the cells of the outside ectoderm have vacuoles that are empty and in others, the vacuoles contain a clear, faintly grayish-stained substance. Often the grayish substance itself is vacuolated. A space appears between the periphery of this substance and the enclosing cell membrane owing to shrinkage. Special staining with toluidine blue indicates that the substance is not mucus, as it does not stain metachromatically.

Some of the sensory anlagen in the outside epithelium have taken on the form generally associated with taste buds. A small cone is made up of cells with wide bases, which be-

come progressively narrower as they reach the distal tip of the cone. The nuclei are basal. Not all the sensory anlagen have assumed this shape, and those that have are not confined to the head region. They are also found on the caudal peduncle.

Cells lining the gill slit are mostly granular, and their eosinophilia is marked. These are closely associated with the cartilages of the gill arches and resemble the chloride-secreting cells of the adult fish.

In the trunk region a thin plate of muscle is seen forming immediately distal to the peritoneum. A small round area of a few cartilage cells is differentiated in the wall and articulates with the proximal end of the pectoral fin cartilage. More anteriorly, a long flat muscle connects the base of the body musculature with the base of the pectoral fin. The muscle spreads laterally immediately dorsal to the transverse septum.

The heart is still open posteriorly to the subdermal space. The pericardial membrane appears continuous with the extreme lateral margins of the sinus venosus; in other words the walls of the sinus venosus at their posterior extremity appear to turn laterally and ventrally to form the pericardium, leaving the sinus lumen wide open to the subdermal space. This area is immediately adjacent to the yolk sac, but in this stage the transverse septum, which forms the dorsal wall of the peritoneal cavity, lies between the abdominal cavity and the subdermal space. The anterior end of the heart tube is open immediately beneath the base of the pharyngeal lining in the midline. The wall of the tube turns parallel to the under side of the pharynx, leaving a narrow lumen which will eventually become the ventral aorta. At this stage it is open ventrally to the subdermal space in an area where mesenchymal cells are becoming numerous. The lumen is closed dorsally.

The posterior part of the gut seems to be distended in almost all the sectioned embryos. The lumen is very large, and the wall is composed of cuboidal rather than high columnar cells, giving a slightly stretched appearance to the tissue. This distended appearance is noticeable also in the photographs of the stained whole mounts (pls. 63, 64). It does not seem normal, as the gut is completely empty; the larvae have not yet started to feed. It is possible that this is an indication of some respiratory or osmoregulatory malfunction which may contribute to the cause of the early death of the larvae. This distension is noticeable in the living embryo as a kind of bubble and was seen in both *Antennarius* larvae that were kept in running sea water and in *Histrio* larvae that were kept in standing sea water.

The size of the pancreas has increased greatly, and the organ now has two lobes. The liver has also increased in size and has almost surrounded what is left of the yolk sac. So little yolk is left that the tough yolk-sac membrane, originally formed by the periblast, is conspicuous. Both yolk and membrane are contained within the boundaries of the liver.

Stage 23

Stage 23 occurs at about 190 to 200 hours or eight days after spawning. Plate 64, figures 1 and 2, are photomicrographs of hematoxylin-stained whole mounts in this stage. Figure 1 shows a larva which is obviously in much better physical condition than that in figure 2. The curved condition of the notochord in the latter is an abnormality common among the larvae that will not survive. A decreased affinity for the stain is also common to the larvae when they are moribund. This can be seen also in the larvae pictured in figures 3 and 4 which survived for another day. The larva shown in figure 1 has a little more yolk material left than the one in figure 2, and its better staining reaction shows the development more plainly. The nasal placodes are clear, part of the heart is obvious, and the jaws are notably large and powerful.

The ventral limit of the infundibulum rests immediately above the bifurcation of the parasphenoid bone. There is still no evidence of a buccal epithelial contribution to form the hypophysis. The anterior end of the notochord reaches forward to the base of the hypothalamic region.

Otoliths are beginning to form from the transverse membrane in the otic capsule.

The outside skin over the head region has become more differentiated. Several layers can be distinguished. Most proximally there is a layer of thin tissue possibly derived from mesenchyme. Next a fibrous layer which has assumed some of the characteristics of collagen is seen. Still farther distad is the layer containing the vacuolated and granulated

cells. The extreme outside layer has small, blunt processes which extend outward into the surrounding medium. This condition is particularly noticeable in the skin over the top of the brain and under the heart; skin covering the trunk region and caudal peduncle is not yet so differentiated.

The muscles of the lower jaw have developed greatly by this stage. These are inserted at the anterior end of the mandible, and the posterior end of the muscle fiber appears to be connected to the base of the pectoral fin. At this stage, the components of the pectoral fin represent the only skeletal parts posterior to the head region.

The heart is divided into four chambers. The sinus is still open to the subdermal space. A distinct narrowing of the walls in one area makes the transition from sinus to auricle. At this point and also between the auricle and the chamber that will become the ventricle, the stricture of the wall of the tube is sharp, and an accumulation of cells within the lumen gives the effect of developing valves. The wall of the developing ventricle again narrows before the tube opens out under the gill arches to form the ventral aorta.

The thymus anlage is represented by a small cluster of deeply staining cells on each side of the larva immediately anterior to the anterior end of the pronephric ducts, where the duct makes its single loop. In frontal sections, the anterior portion of the anlage rests between the posterior edge of the otic capsule and the angle formed by the posterior boundary of the gill cavity. These cells have little cytoplasm, but the nuclei are large, and the chromatin is heavily stained. The organ is surrounded by a heavily pigmented capsule.

So little yolk material is left that the yolk membrane is as conspicuous as the yolk itself within the capsule of the liver (pl. 72, fig. 4). The liver capsule is heavily invested with melanin, as are all the other visceral organs: intestine, pancreas, and renal ducts.

Many of the sectioned larvae appear greatly dehydrated, which could be assumed to be the result of the histological techniques were it not for the fact that some larvae appear normal. When the larvae were embedded in paraffin, several were run up and embedded at the same time, going through identical procedures. When sectioned several larvae were in the block. When seen under the microscope, in a group of four or five larvae, one or two appeared normal and the rest were dehydrated. The only conclusion is that they were in different stages of hydration at the time of fixation.

Stage 24

Stage 24 is the stage at which the greatest mortality occurs. In *Antennarius* under the temperature conditions that obtained at the time, death took place nine days after spawning. The following day a few scattered larvae were still alive, but these succumbed very rapidly. Plate 64, figure 3, is a photograph of a hematoxylin-stained whole mount at nine days, and figure 5 is a photograph at 10 days. The extra day was not accompanied by further development. The clumped condition of the pigment seen in figure 6 is characteristic of a moribund condition.

All the tissues in section appeared dehydrated, with half-pycnotic nuclei. The melanin was clumped in all the sections; the typical appearance of the sectioned melanophore was lost. No further development of organs had occurred.

A very small amount of debris was seen in the intestinal tracts of occasional larvae. The material was impossible to identify under the microscope. It was cellular and seemed to be of animal rather than plant origin—possibly small copepods. The liver still contained a few unabsorbed yolk particles.

HISTRIO HISTRIO

Photographs of early cleavage stages and later stages of *Histrio* embryological development were published by Mosher (1954). Her photomicrographs were taken of larvae developing under conditions of running water with temperature variation between 21° and 23° C. at Bimini. The photomicrographs used in the present report and found on plates 73 and 74 and figures 5 and 6 of Plate 64 were taken of *Histrio* larvae developing in stand-

ing water at a somewhat higher temperature. A 100-watt bulb was hung over the tank which produced sufficient heat to elevate the temperature to approximately 30° C. The rest of the light in the room was provided by "warm white" fluorescent bulbs; no daylight was ever present. The lights were automatically controlled, so that 12 hours of light alternated with 12 hours of darkness. Under these conditions the development of the embryos was faster than that of the embryos described by Mosher, although none lived longer than 10 days, in contrast to the 12 days recorded by Mosher. However, at the time of death the embryos that had been subjected to the higher temperatures were more advanced than either the *Histrio* or *Antennarius* kept in running water under cooler temperatures. A second factor that may have a bearing on the additional development was the presence of microscopic organisms in the tank of standing water, which may or may not have provided some food for the more differentiated larvae. They were seen to go through feeding motions.

Plate 73, figure 1, represents the late blastula, comparable to stage 9 of *Antennarius*. In this particular fertilized raft this stage occurred about 20 hours after spawning. The spawning took place on January 28, 1957. Subsequent observations on rafts fertilized later in the year demonstrated that the early germ ring (pl. 73, fig. 2) and sometimes the embryonic shield stages (pl. 73, fig. 3) would have been developed by 20 hours after spawning. This was undoubtedly a result of increased temperature in the laboratory.

Most of the spawnings took place late in the day. When the young fish first reached maturity, the spawnings occurred between 6 P.M. and 7 P.M. As the fish grew older, they spawned between 5 P.M. and 6 P.M. and later some spawned between 4 P.M. and 5 P.M. By 9 A.M. the following morning, the developing embryos were almost invariably in the late germ ring stage.

Plate 73, figure 4, shows the *Histrio* embryo in stage 14 approximately 36 hours after spawning. The optic vesicles are prominent, and Kupfer's vesicle is formed. The germ ring covers at least three-quarters of the yolk. Peculiar clusters of cells are present in the outside ectoderm which were also characteristic of *Antennarius* embryos. These are seen more clearly in plate 73, figure 5, which represents stage 15. Somites are visible here, and the faint lines streaming out on each side of the somites are caused by developing mesenchymal tissue. Stage 16 is represented in plate 73, figure 6, showing differentiation of the eyes and lenses, developing gill slits, auditory placodes, and increase in the number of somites. This stage occurred approximately 40 hours after spawning. Plate 74, figure 1, is a different view of the same stage, showing the somites, developing mesenchymal tissues, and the extent of the germ ring which does not yet cover the entire yolk area.

Early stage 17 (pl. 74, fig. 2) is reached about two and one-half days after spawning, and late stage 17, when the embryos are hatching, occurs about three days after spawning. The hatching embryos are seen in plate 74, figures 3 and 4. The newly hatched embryo has very little pigment. A few melanophores are seen around the gut. The lenses and optic cups are well formed, but no retinal pigment is present. No change in this pigmentation was noted up to 95 hours. Hematoxylin-stained whole mounts of this stage show that the pectoral fin bud is formed and appears somewhat closer to the body than in *Antennarius*, although it is still connected to the main trunk of the embryo by only a thin thread of cells.

Stage 18 or 19 is represented in plate 74, figure 5. This stage occurred about 116 hours after spawning. *Antennarius* at this time was in stage 19, but the *Histrio* pictured here seems somewhat less developed to judge from the volume of the yolk present. Hematoxylin-stained whole mounts show a considerable increase in pigmentation from the previous stage, particularly around the gut and the choroid region of the eye. Retinal pigment is still extremely scarce; only a faint melanin granulation can be seen in this area. The pattern of pigmentation is slightly different from that seen in *Antennarius*. At the anterior end where the pigmentation spreads out behind the eyes, the pattern of the Y seen in *Antennarius* is not present. Dispersed melanophores fill in the acute-angle area. Melanophores also spread out a little on each side of the stem, over the dorsal surface of the yolk, so

that the pattern is much less sharply defined.

The remainder of the embryo seems to be developed to a degree comparable to *Antennarius* at this stage. The pronephric ducts and small urinary vesicle are present. The otic capsules and nasal placodes are plainly seen, and the pectoral fin buds protrude at a slight angle from the body wall.

The *Histrio* larvae reached stage 21 six days after spawning. The only difference in pigmentation between *Histrio* and *Antennarius* at this time was observed in the retinas, which contained less melanin in *Histrio*. The pattern of *Antennarius* pigmentation at this time has changed from the Y to the more diffuse form. It would seem to be impossible to differentiate between the two species at this stage. After the pigmentation in *Antennarius* has passed through the Y stage, the diffuse pattern is the same in both species. Both have the same broad, embryonic fin fold covered with vacuolated cells in the outside ectoderm, and pectoral fins protrude from the body wall in the same areas. The development of the brain is in the same stage, both species characterized by the large, thin-roofed space of the fourth ventricle over the medullary region. The volume of the yolk is greatly reduced in both. In stained whole mounts, the *Histrio* larvae show a sigmoid curve in the gut, pronephric tubules, and a urinary vesicle somewhat larger than in previous stages. The gill slit is seen to be broken through, and the tube of the heart is twisted near the anterior end. Myomeres have formed from the somites and are found lateral and ventral to the notochord. None of these characteristics are shown exclusively by *Histrio*. All descriptions apply equally well to *Antennarius*.

The living *Histrio* larva in stage 22, seven days after being spawned, is seen in plate 74, figure 6. Pigmentation of the retina is complete at this stage, and the eyes show the forward rotation which is also characteristic of *Antennarius*. The brain is so flexed that it presents a broad aspect in the anterior of the larva, causing the fish to appear as if it had a very high, flat forehead. *Antennarius* larvae were not seen to go through this morphological stage (see pl. 63, figs. 5 and 6). This conformation is a transient one in *Histrio*. The eyes of *Histrio* are smaller than those of *Antennarius* and more nearly spherical. At this and later stages, *Antennarius* eyes appear to be slightly compressed or flattened on the anterior surface.

The fixed and stained embryo shows the mouth to be open and cartilages of the lower jaw and parasphenoid to be well developed. Cartilage could not be identified in the pectoral fins. The otic capsule shows a division into two vesicles. The gut has a double curvature and is distended at the posterior end. No debris is seen within the intestinal tract, and no yolk could be identified. Whatever remains of the yolk is obscured by the pigmentation. Mesenchyme is seen spreading out from the posterior tip of the notochord, arranged radially in a manner suggestive of fin rays. The tip of the caudal fin, which is not in any other way delineated from the embryonic fin fold, is divided into six points. Only one point is seen on the edge of the pectoral fin. In the living larvae these pectoral fins are moved with great rapidity, and their rapid beat can be stopped instantaneously when the larvae wish to arrest their forward movement.

A photomicrograph of a hematoxylin-stained whole mount of *Histrio* in stage 23 is seen in plate 64, figure 5. This stage occurred eight days after spawning. Considerable mortality had occurred between this stage and the previous one. The curvature of the notochord seen here is not normal and is one of the signs of approaching death. The gas distension of the gut is also extreme, and the shrinkage of the embryonic fin fold is thought to be not entirely owing to the treatment with alcohol. Eight points are now seen on the edge of the caudal fin and three points on the edges of the pectorals. In the brain the fourth ventricle under the thin roof is greatly diminished, and the third ventricle is separated from it by a septum. In some stained preparations the urinary vesicle seems distended as well as the gut. This was also noted in *Antennarius* embryos and can be seen in plate 74, figures 3 and 4.

Two of the small *Histrio* larvae in this stage were stained in dilute toluidine blue for demonstration of mucoid tissues. None of the vacuolated or granular cells of the outside ectoderm showed any positive reactions. The only metachromatic staining was seen in the cartilages. Definitive cartilage was well

marked in these preparations by the purple color which was seen in the mandibles, the parasphenoid, and the cartilaginous plate in the pectoral fin. Other small *Histrio* larvae were stained with Herxheimer's scarlet red for demonstration of fat. No positive reaction was seen in the cells of the outside ectoderm. The only positive reaction to the fat stain was a diffuse staining impossible to locate, but somewhere in either the liver or the gut. *Antennarius* sections in this same stage were also stained with toluidine blue with a similar negative reaction in the outside ectoderm. The granular cells were intensively stained, but orthochromatically with toluidine blue. Whatever the function of these cells, they are not engaged in the production of mucus.

Stage 24 of the *Histrio* larva is represented in plate 74, figure 6. This stage occurred nine days after spawning when so great a mortality had occurred that it was extremely difficult to find a living larva in the tank, which had contained hundreds immediately after hatching. These may possibly represent a more advanced stage than any reached by the *Antennarius* larvae before death and are certainly much more developed than the oldest larva recorded by Mosher (1954). The increased development may be the result of higher temperatures or the intake of food particles or a combination of both. The standing-water tank in which the larvae were raised contained a considerable quantity of a fine green alga and was also rich in small protozoans: ciliates and flagellates, copepods and small crustaceans. When studied under the dissecting microscope, the small larvae were seen to go through feeding motions, striking at invisible things, snapping the jaws, swallowing, and accumulating opaque white material in the gut. What they were eating could not be identified even at a magnification of × 30. The small animal forms listed above seemed to be ignored by the larvae. The small fish showed considerable vigor, were properly oriented, and demonstrated controlled swimming motions; the pectoral fins beat rapidly and were used for braking the swimming speed and for turning.

In spite of the obvious feeding behavior, no food particles or debris of any kind could be distinguished in the gut of the stained and cleared larva. Distension of both the gut and the urinary vesicle was still prominent.

Indications of increased development in this stage over the previous one are the appearance of anlagen of the first two dorsal spines. These can be seen, although not clearly, in plate 64, figure 6. They are noted under high magnification to consist of two short, nearly colorless protuberances from the anterior surface of the head, dorsal to the mouth. At their bases is a cluster of deeply stained nuclei immediately beneath the outside ectoderm. The brain shows a slight evagination from the roof of the telencephalic region which may be the pineal anlage. No development of the pineal organ was noted in any of the sectioned *Antennarius* larvae.

On the surface of the pectoral fins, a band of opaque, white, faintly granular material is seen in the living larva over the distal edge of the cartilaginous plate. This is the first indication of a dermal pigment other than melanin, and is undoubtedly guanin. This is dissolved in the fixing and staining process and therefore cannot be identified in the whole mounts. At least five points are seen on the edges of the pectoral fins and eight on the edge of the caudal fin. None of the median fins are differentiated, and there is as yet no sign of the paired ventrals. The parasphenoid cartilage widens laterally at its anterior end, giving a solid base for the mandible to press against.

DISCUSSION

THE ONLY OTHER KNOWN genus of fishes that produces eggs encased in gelatinous material is *Perca*, one of the central acanthopterygians. Parker (1942) describes the release of eggs from *Perca flavescens* into the central ovarian lumen where they are embedded in a gelatinous substance and ruptured through the body wall at spawning by means of an orifice which appears at a point where ovary and body wall coalesce. He describes the eggs as being spawned in an unbroken, ribbon-like, gelatinous mass, the form of which corresponds almost exactly to the form of the ovarian cavity.

Thomopoulos (1953) states that the presence of the male is not necessary for the release of the ova in *Perca fluviatilis*. This author describes the ovary as having a fluid content similar in composition to coelomic fluid. At the time of spawning, contractions of the distended abdominal wall were noted which, together with the pressure of eggs and ovarian fluid, rupture the membrane that closed the genital pore. Up to this time the membrane prevents the entrance of the outside medium into the ovary. The eggs are again described as being laid in a continuous cord or ribbon. A figure is given of the immature ovary, showing the ova arranged in a continuous, convoluted cord. The actual spawning in this species lasts five to 10 minutes, as contrasted with the instantaneous character of the release of the *Histrio* egg raft. The gelatinous material in this instance forms the outermost coat of the ovum, external to the zona radiata, and is filled with small canals.

In *Perca fluviatilis*, Mayenne (1927) describes two layers of follicle cells surrounding the ovarian ova at his stage D. The outer of these probably refers to what has been called lamellar epithelium in the present report. In stage F, which Mayenne describes as the most advanced stage of development in the ovary, the ovum shows the gelatinous layer already elaborated. The figure shows this layer to be between the chorionic membrane and the follicular layer. Therefore, it cannot be a product of the lamellar epithelium as in *Histrio* and *Antennarius*. This author also describes the ovary as being filled with fluid.

Mosher (1954) appears to have been in error when she likened the membranes separating the ova in the *Histrio* raft to the chorion surrounding the perch egg. In the case of the perch, the gelatinous layer may be called a chorion, as it is elaborated by the follicle cells, but the gelatinous membranes in *Histrio* and *Antennarius* rafts are a product of the epithelium lining the ovary and have no actual connection with the ova during their development. The true character of encapsulation of the ova in the raft of *Lophius* was understood by Prince (1891), Fulton (1898), Bowman (1920), and Procter *et alii* (1928), and is similar to that in *Histrio* and *Antennarius*.

Gill (1908) described the egg rafts of both *Lophius* and *Histrio* (*Pterophryne*) as reflecting the form and structure of the ovaries.

Fulton (1898) described the ovaries of *Lophius piscatorius* as being confluent and forming a single, long, flattened tube, the size of which varied with the size of the fish and the season of the year. The ovaries are extremely thin; one which measured over 29 feet in length was only 4 mm. thick, but over 13 inches wide. This ovary contained almost mature eggs. Ovigerous tissue in this species is also found on only one side of the lumen, the ova arranged in a single layer. However, the figures given of ovaries with eggs in an immature stage show ova in various stages arranged in several layers, so that the single layer of ova mentioned above must refer to only the ripe eggs about to be spawned. In the ovary with mature eggs, Fulton described them as already embedded in a thick gelatinous matrix. No free fluid was found in the ovarian lumen. The ovigerous tissue takes the form of lamellae, as described herein for other pediculates. The inner wall of the ovary contains many lymph sinuses, and the figures published resemble the ovary of *Ogcocephalus* in this regard rather than ovaries of *Histrio* or *Antennarius*. Fulton recognized the gelatinous material of the raft as a secretion product of the epithelium lining the ovary and found it to be present only in ovaries coming in to spawning condition, although he concluded that the lining of the non-ovigerous wall did not participate in the elaboration of mucin

formation in *Lophius*. Fulton described a goblet cell appearance of the lamellar epithelium at the time of mucus formation, but this was not seen in *Histrio*. There is a possibility that such a stage in mucus formation had passed in the ovulating *Histrio* ovary that was available for study for this report.

Gill (1908), who described the eggs of *Histrio*, speculated that *Antennarius* discharged their eggs in a jelly-like envelope or raft and based his belief on "habits and methods derived from a common ancestry." He thought it probable that the mature eggs from one ovary at least were all spawned at once. He said that the act of spawning of *Histrio* was observed at Woods Hole by Hugh Smith in 1897. In as much as the eggs were not fertilized, it seems most likely that what was observed was release of the unfertilized raft by females. The idea that development and maturation of ova take place at different times in the two ovaries seems to stem from an attempt to justify the frequency with which the egg rafts were delivered, although in both cases mentioned by Smith (1898) the releases of the rafts were at least a month apart. Gill described the *Antennarius* egg raft as being 3 to 4 feet long by 2 to 4 inches in breadth, tapering abruptly and blunt at the edges, and having several irregular layers of eggs. No mention was made of the scrolled shape. Other investigators (Gudger, 1937) have seen the release of rafts from captive *Histrio*. Smith (1898) made the first of such observations. Hornell (1921) described the raft of *Antennarius hispidus*. The size of the raft was $9\frac{1}{2}$ feet by $6\frac{1}{4}$ inches. Mosher (1954) published the first account of actual courtship and mating with the production of a fertile raft of *Histrio*.

The egg raft of *Lophius* has been known for a long time, because of the frequency with which it is seen in northern waters and because of its enormous size. The curiosity of various early investigators was aroused and consequently there are numerous references concerning the embryology of these fish. Portions of rafts were picked up at sea and more or less carefully collected or preserved. The embryology consists mainly of descriptions of the external morphological features. Evidently the larvae of this species are hardier than those of *Antennarius* or *Histrio*, for several investigators were able to raise them through an early post-larval stage (Lebour, 1925; Williamson, 1909; Bowman, 1920; Prince, 1891; and others) after they were collected as parts of disintegrating rafts or free larvae. The external characters of these larvae, especially their extensive pelvic fins and modifications of the first dorsal rays, make later stages collected in plankton easy to distinguish. Therefore the life history of *Lophius* from the egg through the post-larva to the young bottom-dwelling form is fairly well known, at least from the point of view of external morphology.

The two most useful of the early references are those of Bowman (1920) and Prince (1891). According to Bowman, the raft released by *Lophius*, while of great size (25 to 30 feet in length by 2 to 3 feet in width) contains only a single layer of ova enclosed by a mucoid envelope. Each ovum is enclosed in a separate capsule, separated from the others, and each can revolve within the fluid enclosed in the capsule. Unlike that of *Antennarius* and *Histrio*, the yolk of the *Lophius* ovum, although mostly homogeneous, contains a few oil globules which later coalescesce into one. The mucus band is described as a light violet-gray in color.

The hatched larvae differ in a few ways from those of *Antennarius* and *Histrio*. These are the simultaneous appearance of fin buds of both pectoral and pelvic fins and the early differentiation of the first three dorsal rays. The larva is much longer (7 mm.) at the time when the yolk sac is absorbed. The transitory appearance of violet or mauve pigment in the gut region described by Bowman (1920) is lacking in the two forms under consideration here.

Prince (1891) studied the embryology of *Lophius* from a few specimens, one of the embryo near the hatching stage, and others immediately after hatching and at five, nine, and 15 days of age (presumably days after hatching). These specimens were taken off the coast of Scotland and apparently were different from those forms described by Agassiz (1882) for the American species. Prince noted the large subdermal space, which he called "subepidermal spaces." The larvae are much more deeply pigmented than those of *Histrio* or *Antennarius*, with many more me-

lanophores on the yolk surface and particularly around the oil globule. On the fifteenth day these larvae must have been much more advanced than any stage reached by *Histrio* or *Antennarius*, as the author mentions the existence of arteries and veins, a cartilaginous rudiment of the neural arch, a pronephros with the addition of a paired Malpighian capsule with glomeruli, and rudimentary gonads.

Padmanabhan (1957) has recently published an account of the early stages of development in *Antennarius marmoratus*. In general, his observations coincide with those reported herein, but there are some notable exceptions. The speed of development was greatly increased over that of both *Histrio* and *Antennarius* described in the present report. The blastoderm was described as covering the yolk by 12 hours after spawning, and the optic cups were formed by 17 hours. Hatching occurred at 22 hours. In as much as the author does not mention at what temperature this development took place, it is not possible to know whether the increased rate of development is a real difference or not. Other differences are a blastodisc raised well above the surface of the yolk, the presence of dorsal aorta, posterior cardinal vein, and other small blood vessels in the larva at hatching, and the presence of blood corpuscles at 48 hours when the yolk sac was not fully absorbed. Most of these larvae were reported as having died at 48 hours after hatching, but some lived to 78 hours, and the latter appear to be in approximately the same stage of development as the oldest *Antennarius* and *Histrio* larvae described for the present report, although the mouth and anus were not developed. The pectoral fins may have been slightly more advanced, as the author reports the appearance of eight or nine fin rays. The adult *Antennarius marmoratus* were taken in floating Sargassum weed and spawned after being transferred to tanks, the egg rafts being entangled in the weed. The eggs are described as being embedded in hollow, tubular, gelatinous masses 18 to 20 inches long, the eggs arranged in the walls in a close spiral. Padmanabhan concluded that the structure of the internal epithelium of the ovary indicates that the gelatinous material is secreted by it.

Marine pelagic embryos are extremely interesting from the point of view of their physiology. The absence of vitelline circulation, capillary beds, and erythrocytes raises the question of how the developing larva gets necessary nutritive elements and oxygen to the tissues. The function of a large subdermal space between the outside ectoderm and the main body of the larva is as yet unknown. Are the fluid contents of the larva at this time in isotonic equilibrium with the surrounding sea water? If not, osmotic work is being carried on in spite of the fact that the kidneys are not yet very far advanced.

Some of the essential differences between pelagic and demersal embryos have been known for a long time. M'Intosh and Prince (1890) have devoted considerable space to a comparison between demersal and pelagic marine ova. Demersal eggs, as compared with pelagic ones, were considered to have a denser capsule and an adhesive coat. These authors considered the adhesive properties to be due to a secretion formed in the oviduct and expelled with the eggs. They also noted the lack of a true stomadeum and proctodeum. Both fissures are produced by a slit of the outside membrane without any invaginations of external ectoderm. They also noted that in gadoids, pleuronectids, triglids, and other pelagic forms, no yolk circulation is ever developed, and no blood-forming islands appear on the yolk surface. The pericardial chamber opens to the subdermal space. These authors thought that blood corpuscles were originally derived from the periblast. Ventral fins were described as late in forming in the pelagic species, appearing generally in a post-larval stage. On the other hand, Agassiz (1882) described *Lophius* as having both pectoral and pelvic fins at hatching, as well as a separation of anterior and posterior dorsal fins, and the figures published by Dodds (1910) confirm this.

References concerning the morphogenesis of pelagic fishes are rare in the literature. Meek (1924) has figured the development of the cod (*Gadus callarius*) up to the time of hatching, which occurred on the twelfth day. He describes one vitelline vein recognizable on the eighth day. The cod embryo also differentiates pronephros more rapidly than *Antennarius*, in as much as tubules and a glomerulus are described as seen on the ninth day. Blood cells were also identified and were derived from the caudal and cardinal veins.

The sinus venosus was described as being "not well defined from the yolk sac sinus," but the Cuverian ducts were identified and found to enter the sinus venosus. The thyroid anlage was also identified on the twelfth day. At this time the yolk sac was still quite large and was attached to the endoderm and splanchnopleure. All cranial nerves were established at this time, and the lateral-line nerve was connected to five pairs of sense organs on the sides of the body.

In describing the embryology of the sea bass, *Serranus atrarius*, Wilson (1889) postulated that the liver absorbs the yolk and identified the cellular capsule which is also absorbed by the liver as the remains of the periblast. A large subdermal space was also described for this species, called by Wilson the body sinus. The space was filled with a gelatinous fluid which coagulated into a loose, stringy mass, with an irregular radial arrangement. These characteristics were not developed until after hatching, although the subdermal space was apparent in just-hatching embryos. The cavity of Kupfer's vesicle was seen to lie between endoderm and periblast, termed by Wilson the terminal part of the archenteron. As does that of *Histrio* and *Antennarius*, the *Serranus* larva appears to be provided with special cellular arrangements in the outside ectoderm, although from Wilson's figures these are much simpler in construction. The outside ectoderm was described as being composed of three layers: an extremely thin squamous layer of cells on the outside and two strata of "nervous layer" cells. The middle of these layers was shown to be highly vacuolated.

The amount of light reaching the embryo has a great influence on the time of melanin appearance. In both *Histrio* and *Antennarius* the black granules identifiable as melanin are seen in melanophores which have probably migrated from the neural crest region ventrally to lie on each side of the gut. In *Histrio* raised in the laboratory, melanization was not noted until after this migration had taken place. In one raft, which had been cut in two and each half placed in separate tanks, pigment cells made their appearance around the gut region in that part of the raft that had been maintained under a 100-watt lamp during 12 hours of the day. Pigment did not make its appearance until some hours later in the half of the raft kept in the adjacent tank, subjected only to the normal fluorescent lighting of the laboratory for the same time periods.

Recognizable melanin was seen in *Antennarius* embryos at a much earlier stage, before the melanophores had migrated ventrally. This was probably owing to the greater amount of light available in the Bimini laboratory where there is a skylight directly over the tanks. In this case, also, pigment appeared in a part of the raft kept in a plankton net floating off the dock before it appeared in the embryos of a part of the same raft kept in the inside laboratory, although in later development, under less light, the quantity of pigment eventually elaborated does not seem to have been affected. The figure in Mosher (1954) that represents the *Histrio* larva at hatching under conditions of light obtaining at Bimini shows a much greater quantity of pigment than larvae raised in the New York laboratories under artificial illumination, although at the time of death the pigmentation in the two groups appears to have been about the same.

Shelbourne (1946b) has recently published some interesting observations on pelagic larvae. He has divided fish eggs into three categories: pelagic marine, demersal marine, and demersal fresh water. Pelagic marine embryos are characterized by large, inflated, marginal fins and absence of vitelline circulation; demersal fresh-water embryos are characterized by "ill-developed" marginal fins and good vitelline vascularization; demersal marine embryos lie between these extremes. While this statement holds true for many cases, unfortunately it is not possible to categorize so neatly all fish embryos. Meek (1924) clearly showed a single large vitelline vein in the pelagic cod embryo. Orton (1957), through a wide acquaintance with numerous teleost embryonic marine forms, points out that several species, mainly among the synentognaths and the allotriognaths, have prominently developed blood vessels in the yolk sac. This author does confirm Shelbourne's statement that those embryos with a vascularized yolk have more moderately developed median fin folds than do those in which the yolk is unvascularized.

Shelbourne (1955) has pointed out that the pelagic embryo has large subdermal spaces in contrast to the embryos of demersal eggs in which the outer epithelium is closely attached to the body mesoderm. These subdermal spaces eventually become confluent with the yolk-sac sinus, and it is Shelbourne's idea that nutritive yolk materials are present in the fluid in these sinuses, providing nutriment directly to the tissues without first being assimilated by the blood stream. This author (1956b) discusses the necessity for osmotic work on the part of the marine embryos if they are not to become desiccated. The question of whether or not the chorionic membranes of marine ova are permeable to sea water has not been definitely settled and appears to be different in different species. In as much as this point has been thoroughly discussed by Shelbourne, it need not be repeated here. However, once the embryo is hatched, whatever protection was afforded by the chorionic membrane is lost, and unless the fluid contents of the newly hatched embryo are in isotonic equilibrium with the surrounding medium, osmotic work must be carried on by some means. In the same paper, Shelbourne points out the necessity for both hatched and unhatched larvae to maintain sufficient water to preserve their buoyancy. This fluid, he thinks, is provided by the yolk diluent received from the ovary at maturation of the egg, and collected in the subdermal spaces of the larva as its nutrient materials are withdrawn by the developing tissues. Emery (1883) believed that the subdermal space was filled by an inward secretion of the skin.

The problem of doing osmotic work in larvae of pelagic marine fishes is probably solved in more than one way and probably differs in different species. Sumwalt (1928) showed that in the developing *Fundulus* embryo, the chorion plus the skin of the embryo were more effective against permeability than the chorion alone. In *Antennarius* and *Histrio* larvae, speculation centers about the peculiar groups of cells in the outside ectoderm and the early development of granular, eosinophilic cells, resembling adult chloride-secreting cells, in the gill membranes. If these cells are engaged in excreting salt and thus doing osmotic work, it is certainly not a characteristic of all marine pelagic larvae. Wilson (1889) mentions only the vacuolated cells in the outside ectoderm of *Serranus atrarius*, and Meek (1924) mentions neither type of cell in embryos of *Gadus callarius*. Although these investigators were not familiar with the concept of chloride-secreting cells, it seems impossible that they would have missed such obvious structures had they been present. No such ectodermal characteristics were mentioned by the early investigators who described developing *Lophius* larvae, and here also, in this closely related species, it is difficult to believe that the structures would have been missed by investigators who paid such close attention to external morphology.

Aside from osmoregulation, other problems are raised by the divergence of the embryology of pelagic fishes from the classic pattern of the fresh-water demersal forms, which is so much better understood. Sections of *Antennarius* embryos up to the time of their death show not only a lack of vitelline blood vessels but an almost total lack of vascularization. The heart pumps the fluid of the yolk-sac sinus, the fluid entering through the sinus venosus which is open to the yolk-sac sinus, and leaving from an ill-defined bulbus into the same sinus. Portions of the dorsal aorta are established but are not connected to the heart, and nowhere are blood cells evident. Unless hemoglobin is dissolved in the fluid of the yolk-sac sinus and subdermal spaces, it is absent. If it is present, it is not in sufficient quantity to give any color to the embryo anywhere. Only a study of older larvae as they approach metamorphosis can give a clue as to where and when blood cells are formed. Rasquin (1955) noted a lack of hemoglobin-bearing cells in leptocephali of *Albula vulpes*, although the vascular system was fairly well established. As metamorphosis progressed in this species, hemopoietic tissue was developed between the kidney tubules, and the characteristic color of hemoglobin became obvious in the circulating blood cells, giving a reddish hue to the regions of the heart and gills. *Albula* leptocephali, when caught, may measure as long as 60 mm. which represents a much greater size of animal living without hemoglobin-bearing cells than the larvae of either *Antennarius* or *Histrio* or many other warm-water forms. Leptocephali of eels and

morays are of much greater size than those of *Albula*.

The assumption that nutrient yolk material is delivered to the fluid of the yolk-sac sinus and subdermal spaces cannot be seriously disputed. However, the yolk-sac membrane becomes increasingly thicker and tougher as the yolk material decreases in volume. Without evidence to the contrary, this membrane would seem to offer considerable resistance to dissolution of yolk material or transport of the same material through its wall. Cells of the liver rest directly upon the yolk material without intervention of the yolk-sac wall, and yolk material can be identified within the liver cells. Even the heavy yolk-sac membrane is eventually incorporated within the liver. It is obvious that many of the yolk nutritive elements are absorbed by the liver, but in the absence of a vascular system, it is doubtful that these are redistributed to other developing tissues. Therefore, nutrient yolk material must be delivered to the fluid of the yolk-sac sinus through the yolk-sac wall before it is completely incorporated within the liver. Daniel (1947) has reported three main centers of glycogen concentration in salmon embryos in liver, muscle, and cells lining the yolk sac. In the fertilized egg, the perivitelline space was described as having a strong positive reaction to stains for glycogen. Daniel considers that the glycogen demonstrated in the liver in early embryonic stages in *Salmo* is not obtained directly from the yolk. The developing embryos of this species have a vitelline circulation, and, although the liver is intimately associated with the underlying yolk, the liver capsule separates the two. This is not the case in *Antennarius* or in *Serranus* (Wilson, 1889).

Many questions remain unanswered, because the untimely death of the larvae intervenes before sufficient development occurs. Thus the whole question of the endocrine glands, such as the formation of the hypophysis and thyroid derivation, gonad development, and the appearance of islet tissue, are all undescribed. The manner of blood-cell formation is unknown; no islands of blood-forming tissue appear on the yolk surface in these larvae. The answers to all these questions depend upon the raising of pelagic larvae in captivity. Various attempts have been made along this line, but no substantial success has been achieved. The best results have been produced by the use of embryos that are fairly large at hatching, but even then the yield is so small that significant embryological results cannot be obtained.

The difficulty is not entirely that of feeding the young larvae. The most critical period, nevertheless, seems to be that point at which the yolk is completely absorbed and the larva must begin to subsist on extrinsic material. With some forms, it seems fairly obvious that lack of nourishment is the critical factor in their early death. Thus, although the young *Histrio* larvae were observed to go through feeding motions, and in some cases an accumulation of opaque white material was seen in the gut, they evidently did not get sufficient nourishment. Very little debris was seen in the gut of sectioned *Antennarius* larvae, and none was seen in the cleared and stained *Histrio* whole mounts. This may have been simply a result of not having the proper food available. However, under the same circumstances, in this laboratory, *Bathygobius* larvae were seen to feed, and sections showed the intestinal tracts to be filled with food, yet the same mortality took place. Tavolga (1950) reported feeding behavior in *Bathygobius* larvae, but he was unable to raise them to metamorphosis. The larger size of the *Pleuronectes* larvae enabled Shelbourne (1956a) to feed them with *Artemia nauplii*. In this instance the hatched larvae were as much as 6 mm. in length.

The *Histrio* larvae used for the present report were carried to a stage of development in the laboratory in standing water further than were those maintained in running sea water. The standing water supported a culture of various kinds of Protozoa and algae which the clear, running sea water at Bimini does not contain to nearly the same extent. This raises the question of what these fry find to eat in nature. If one assumes that they are hatched in the Sargassum weed in which the egg raft may be entangled, there may be an abundance of small food particles, for the weed supports the existence of small barnacles, hydroids, and bryozoans. However, these in turn could feed upon the larvae. This still does not answer the question for other spe-

cies, of which the larvae are adrift in the open ocean. It is also questionable whether the *Histrio* egg raft does indeed remain within the weed. Probably many larvae die at the critical period, even if they do not form food for other organisms, but enough survive to keep the sea abundantly supplied with species that are not by any means on the way to extinction.

It is possible that osmoregulatory difficulties play an important role in larval mortality. Shelbourne (1956a) found that *Pleuronectes* larvae sickened when feeding rendered necessary a change of water in standing tanks. It is certainly true that sections and whole mounts of late *Antennarius* larvae appeared to demonstrate a desiccated condition, which was not a result of histological techniques, although the larvae were kept in running water and presumably were not subjected to changes in salinity.

Other factors have been cited by various authors as influencing the mortality of fish larvae. Blaxter (1956) stresses the importance of constant temperature. Dannevig and Dannevig (1950) emphasize the importance of the gas content of the water. Small bubbles of gas attached themselves to the tiny fry of cod and herring, and they then turned white and died. Herring larvae kept in aquaria often developed gas bubbles in the intestines, and after some time these larvae floated to the surface and died. The authors claim that this phenomenon is the result of supersaturation of the water with atmospheric gases. They found that older fish living in a water reservoir 4 meters deep did not generally suffer from a gas disease, while fish living under nearly atmospheric pressure in shallow aquaria often suffered from a gas disease, although both types of aquaria were supplied with water from the same reservoir. In cod fry, the gas gland was found to function too actively for the pressure under which the fry live in captivity, and the swim bladders burst. In the opinion of these authors, the fatal effect may result from keeping the fry at too low a pressure when the gas gland begins to function and they cite the fact that cod larvae are most commonly collected at 10 to 30 meters in depth in the Skagerrak.

In *Antennarius* and *Histrio* larvae at the time of their greatest mortality there is no sign of the swim bladder. However, it is possible that the gas noted in the intestinal tract may be due to either supersaturation of the water with gases or too low a pressure. The running sea water in the tanks in the New York laboratory were provided with an aerator, but it is doubtful if the water contained as much gas as that in Bimini. No one yet knows at what depth either of these larval forms is found, so that it is possible that the aquaria only a foot deep are unable to provide suitable pressure.

Morris (1956) reviews the literature pertaining to abnormalities in larvae raised under different conditions of temperature. He thinks that temperature must be controlled within very narrow limits, and his best results were obtained when the temperature was controlled within 0.25° C. While most of the abnormalities noted among developing larvae relate to easily distinguished meristic characters, Morris concludes that other organs the functions of which are purely physiological could be affected during development by adverse temperature and other ecological conditions, and that such abnormalities may make their appearance when the yolk has been completely absorbed. He notes a hydrophobic surface of marine larvae to which bubbles easily adhere and thinks this condition may be indicative of some protective mechanism against the desiccating effect of salt water, in as much as it is not a problem with freshwater fish larvae. This author contends that the critical period involves a combination of factors such as a change not only in nutriment but in respiration and osmoregulation as well. The organs necessary to carry out these vital functions must have developed to their proper activity by the time they are called on to function. Gills and alimentary and renal tracts must provide coordinated activity.

Antibiotics and increased knowledge of nutritional factors, together with the development of micromethods in biochemistry of the present day, should be able to solve some of these problems. It is to be hoped that some investigators versed in these techniques will turn their attention to this field.

SUMMARY

THE HISTO-MORPHOLOGY of the ovaries of the pediculates *Histrio histrio*, *Antennarius scaber*, and *Ogcocephalus vespertilio* is described.

2. The ovaries of all three species show similar characteristics: fusion of the two glands at their caudal ends, presence of ovigerous tissue on one side of the lumen only, and considerable quantities of smooth muscle in the walls.

3. The ovaries of *Histrio* and *Antennarius* consist of flattened sacs scrolled from the distal lateral tips ventrally towards the midline of fusion; the ovary of *Ogcocephalus* is scrolled along the longitudinal axis of each ovary, the long edges of the organ rolled ventrally.

4. The structure of the egg raft of *Histrio* is described. The mucoid material in which the ova are embedded is secreted by the epithelium lining the ovarian walls and covering the lamellae. At ovulation, the mucoid material is cast into the lumen along with the ova which have ruptured from the follicles. The form of the raft is a replica of the internal surfaces of the ovary.

5. Pores in the raft are formed by spaces caused by the junction of lamellae with the ovarian wall where no mucus-producing epithelium is present. When the raft is released, sea water enters the pores, presumably carrying sperm with it. Each ovum is then confined in its own mucoid-walled compartment, free to revolve in the sea water contained within the compartment.

6. The frequency of raft release by mature female *Histrio* was found to vary between once every three days and intervals of several weeks.

7. The explosive character of the release of the raft is discussed. The central portion of the raft, from the fused part of the ovary immediately above the oviduct, issues from the vent first, followed by the portion from one side of the ovary, followed in turn by the portion from the remaining side. The ovulation takes many hours to complete; the final release is affected by pressure of the mass within the ovary, contractions of the muscular ovarian wall, and contractions of the abdominal muscles.

8. A detailed description of the early embryology of *Antennarius* is given, with a short comparison with *Histrio* development. Development of these pelagic larvae shows several deviations from the classic pattern of vertebrate embryology. Up to the time of their death, nine days after having been spawned, the following features were noted to be lacking: vitelline circulation, hemoglobin and blood cells, and nephric tubules. No division of somites into somatopleure and splanchnopleure could be identified. No true proctodeum or stromadeum is developed, as there is no invagination of outside ectoderm in these areas. A simple slit appears in the outer covering. The yolk is absorbed by the liver.

9. The larvae possess large subdermal spaces and specialized structures in the outside ectoderm. The possible relationship of these structures to osmoregulatory functions is considered in the Discussion.

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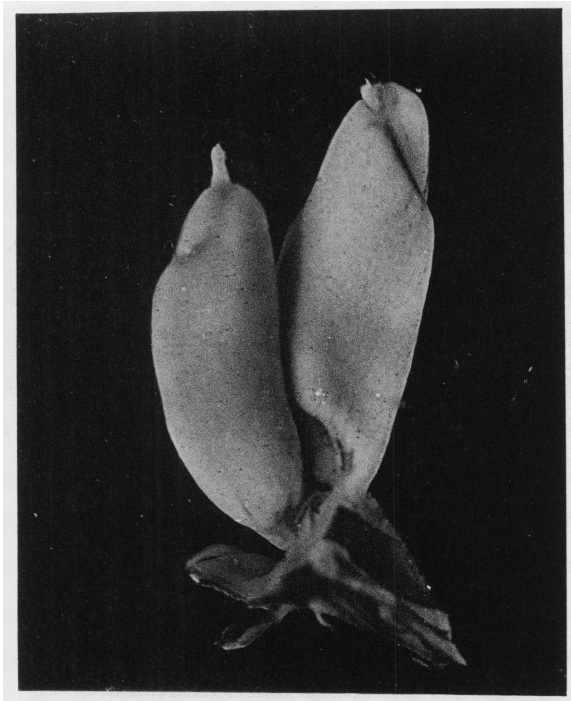

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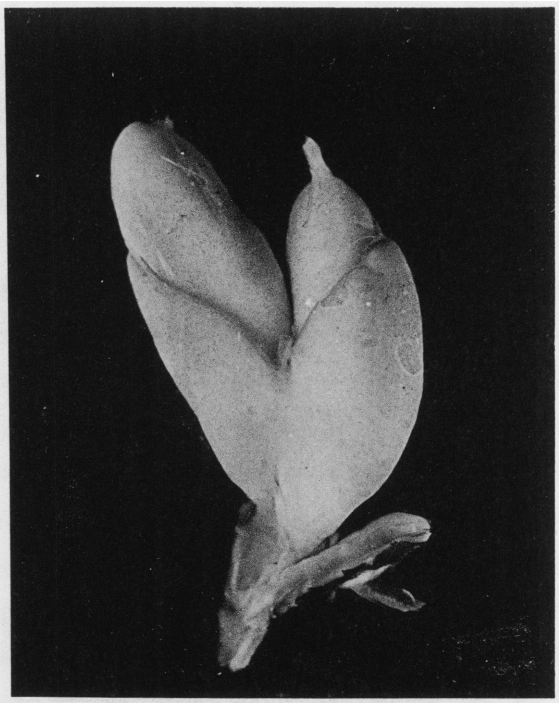

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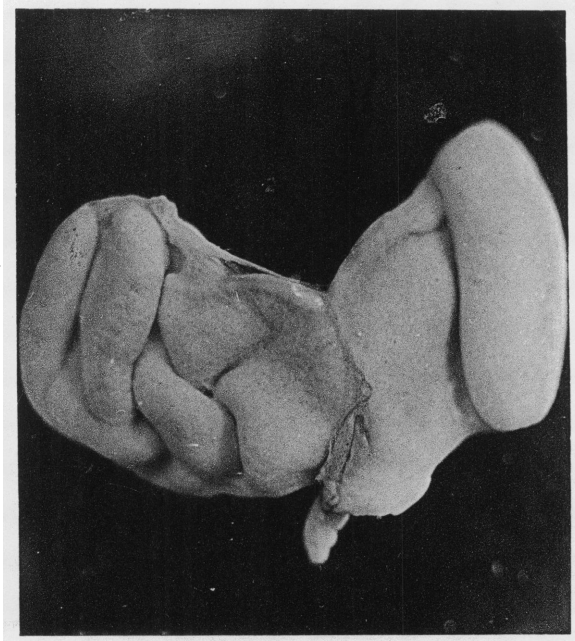

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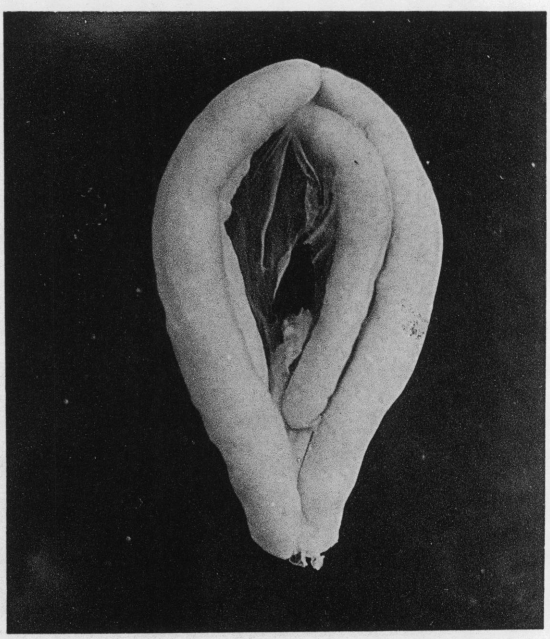
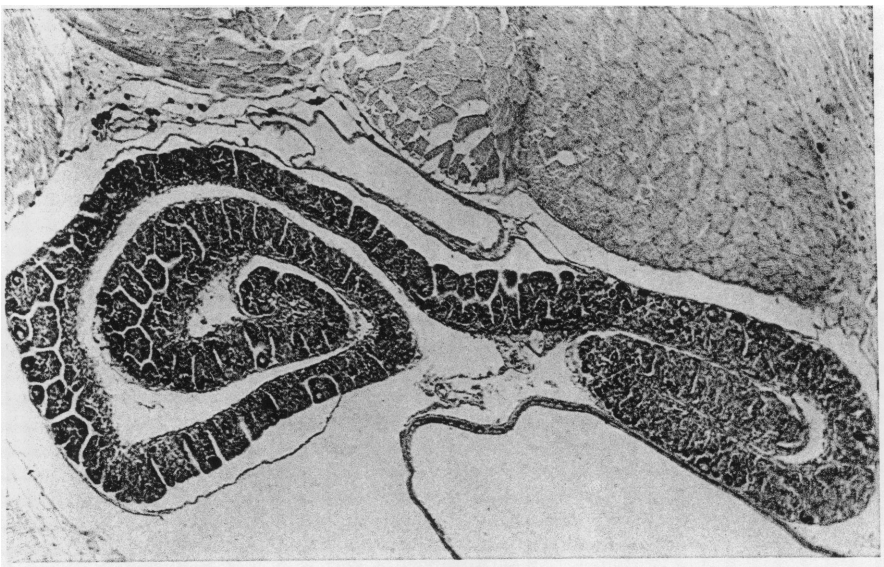


4

1. Ventral view of dissected *Histrio* ovary 13 days after spawning. × 3
2. Dorsal view of same *Histrio* ovary 13 days after spawning, showing fusion in middorsal line. × 3
3. Ventral view of dissected *Histrio* ovary a few hours after spawning. × 2
4. Ventral view of *Ogcocephalus* ovary which contained no ripe ova. × 2



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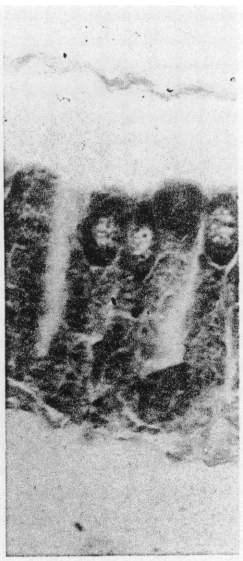

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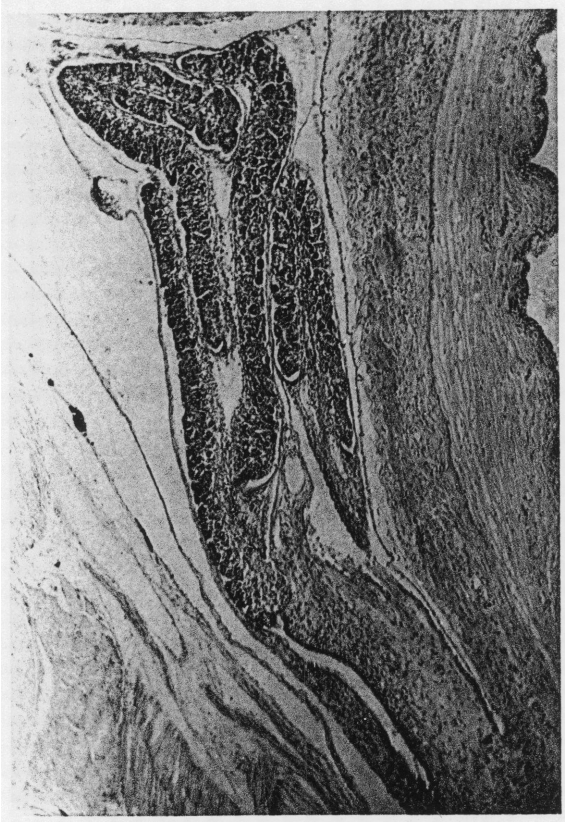

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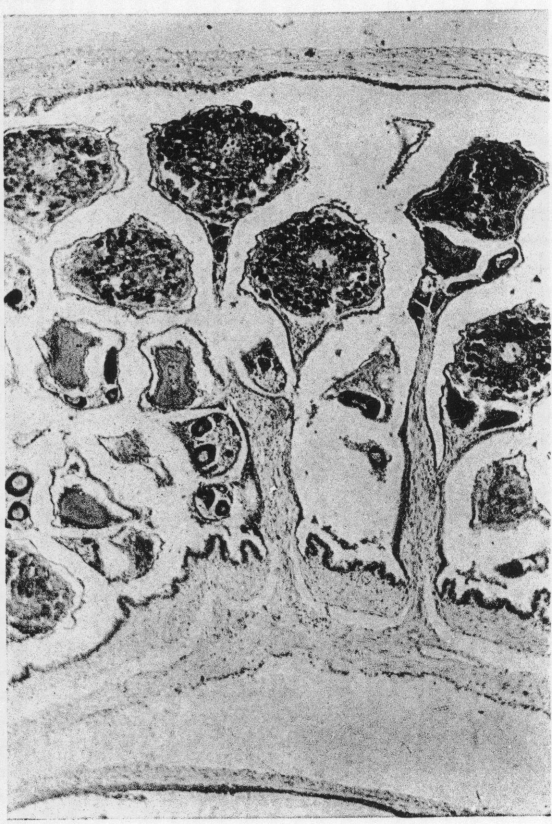
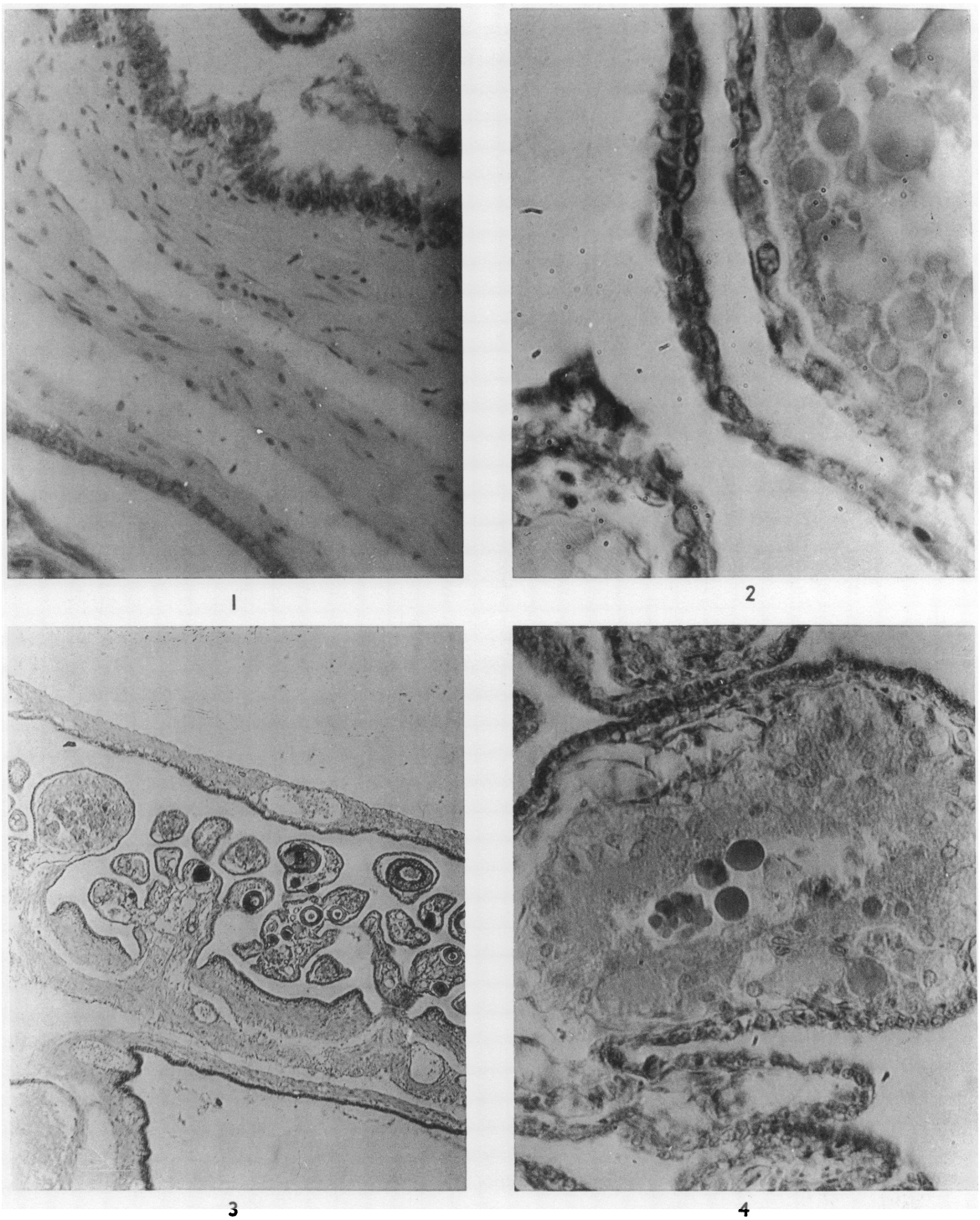


4

1. Transverse section through immature *Histrio* ovary, showing characteristic double scroll. × 85
2. Detail of 1, showing ovigerous lamellae on inner side of lumen and non-ovigerous opposite wall. × 450
3. Longitudinal section of immature *Histrio* ovary, showing continuity of ovarian lumen with that of oviduct. × 85
4. Transverse section through mature *Histrio* ovary 13 days after spawning, showing arrangement of ova, lamellae, and stroma. × 160

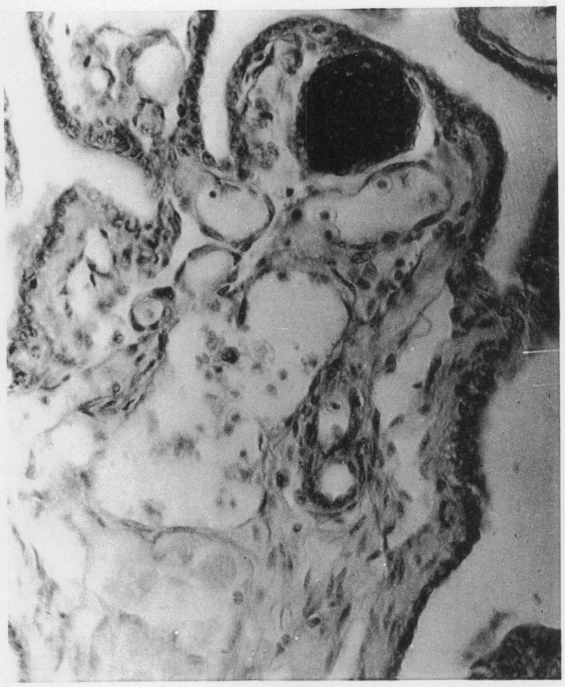


1. Detail of plate 48, figure 4, showing stroma of mature *Histrio* ovary in coiled area where two sides of sac adjoin. Cuboidal epithelium at lower left covers connective tissue on ovigerous side of lumen; columnar epithelium at right covers non-ovigerous wall. × 450

2. Detail of same ovary, showing three layers of cells surrounding mature ovum. Chorionic membrane is represented by the clear band between follicle cells and yolk. × 1100

3. Transverse section of *Histrio* ovary six days after spawning, showing increased vascularization and lack of ripe ova. × 85

4. Detail of 3, showing proliferation of follicle cells engaged in resorbing yolk of an unovulated egg. × 450



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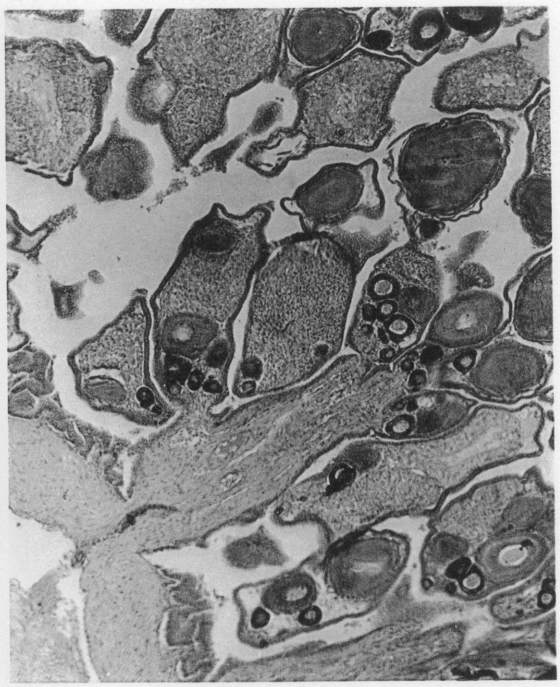

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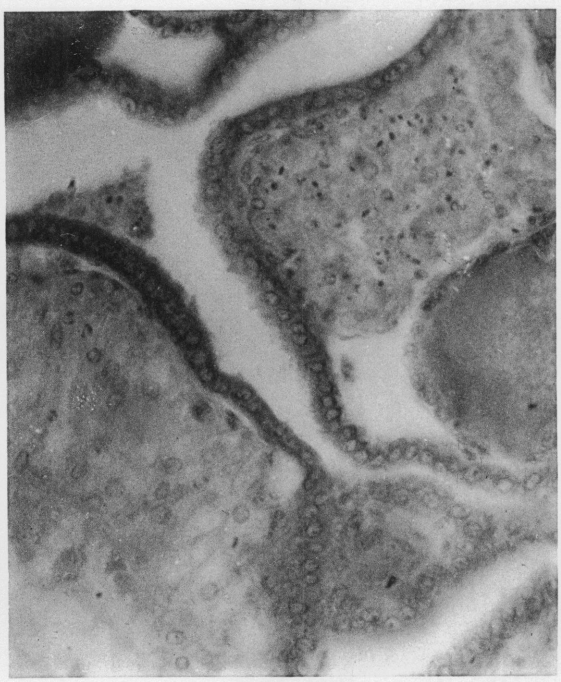

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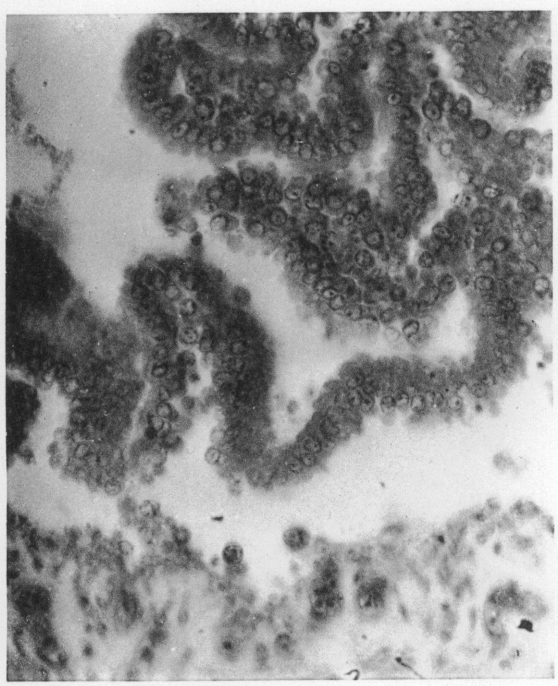


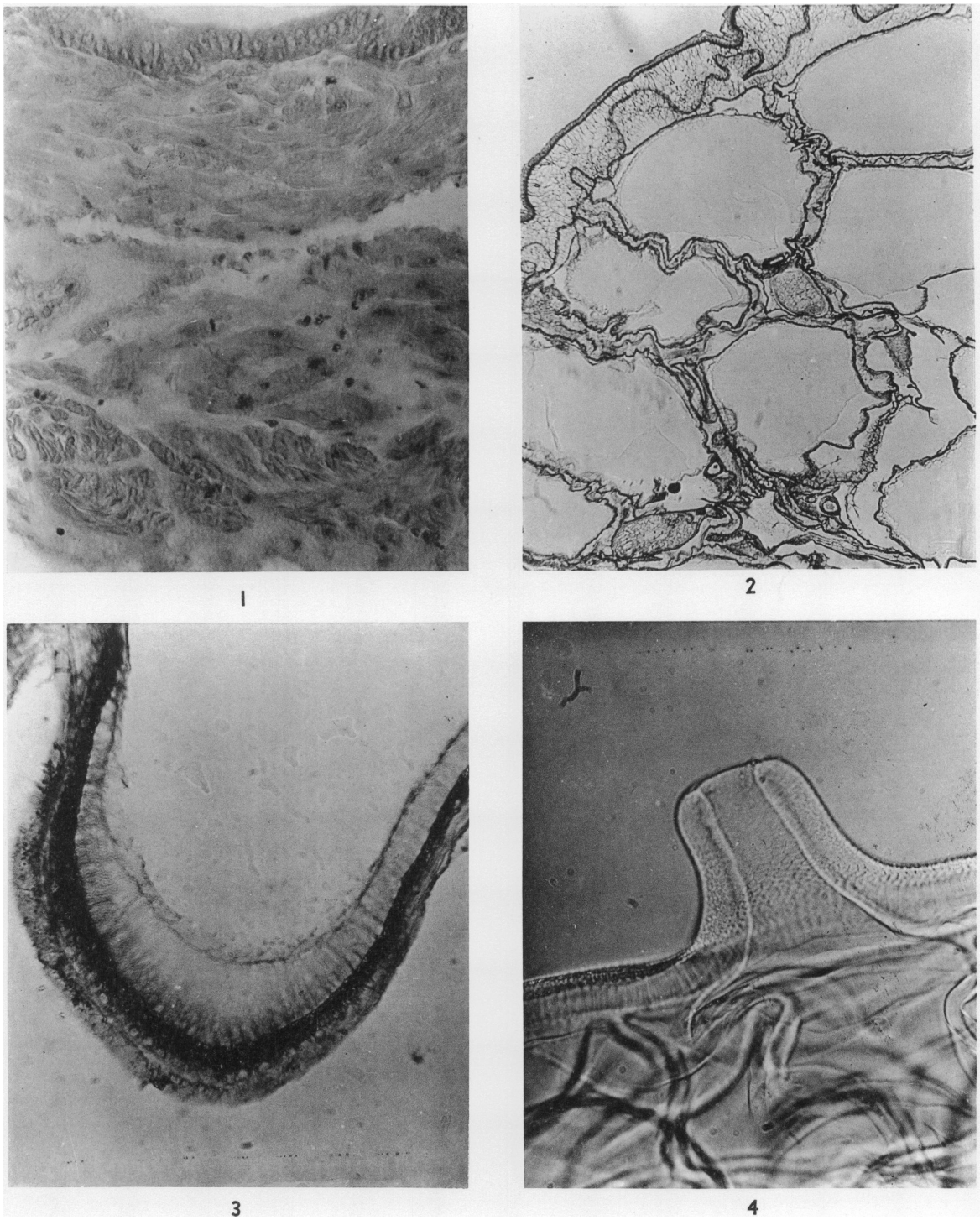
4

1. Detail of ovarian lamella from *Histrio* ovary six days after spawning, showing increased vascularization. × 450

2. Section through ovary of *Histrio* which had spawned only a few hours previously, showing many unovulated eggs. × 160

3. Detail of 2, showing two unovulated eggs in different stages of resorption. × 450

4. Detail of same ovary, showing multiple folds of lining of ovarian lumen. × 450

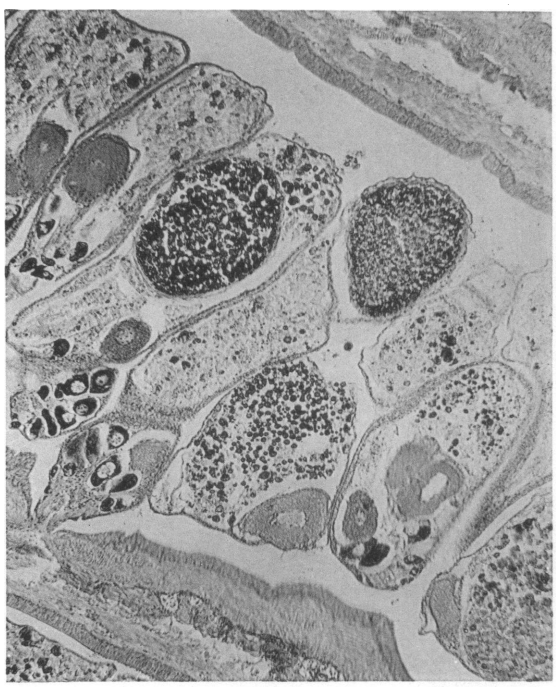


1. Section through inner wall of ovary of *Histrio* a few hours after spawning, showing collagenous and smooth muscle fibers. × 450

2. Section through ovulating ovary of *Histrio*, showing production of mucoid material of raft by epithelium of the lamellae and lining of the lumen. Ripe eggs are unstained. × 160

3. Detail of wall of ovulating ovary showing stretched condition of stroma and mucus being exuded from epithelial lining. Dark band represents epithelium; the mucoid material is above it. × 450

4. One of the pores found on inner surface of scroll of released egg raft, through which sea water enters



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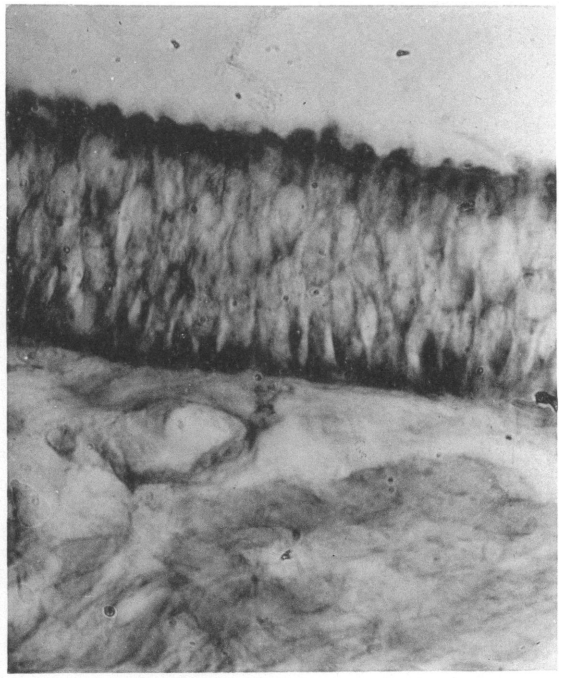

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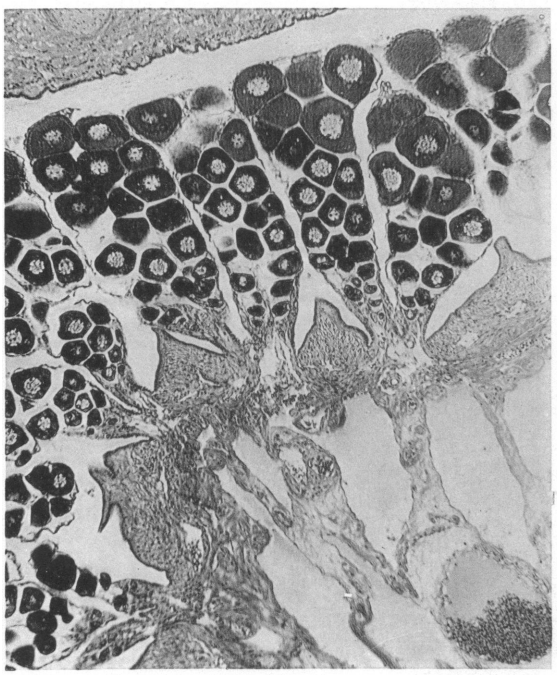

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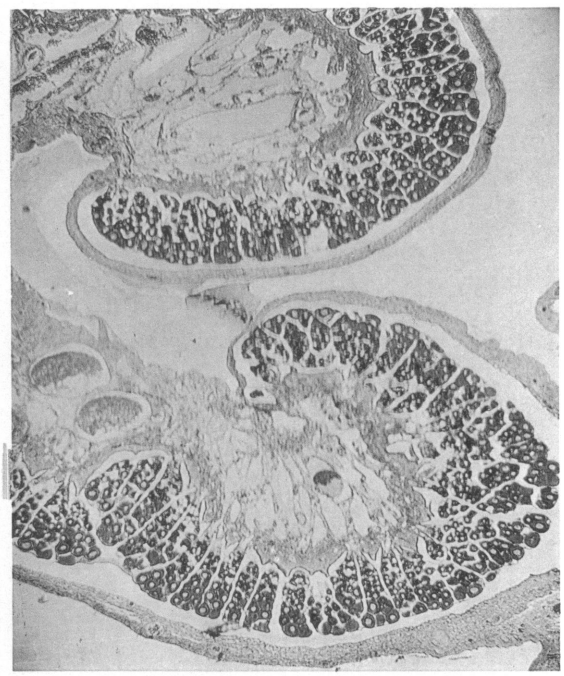


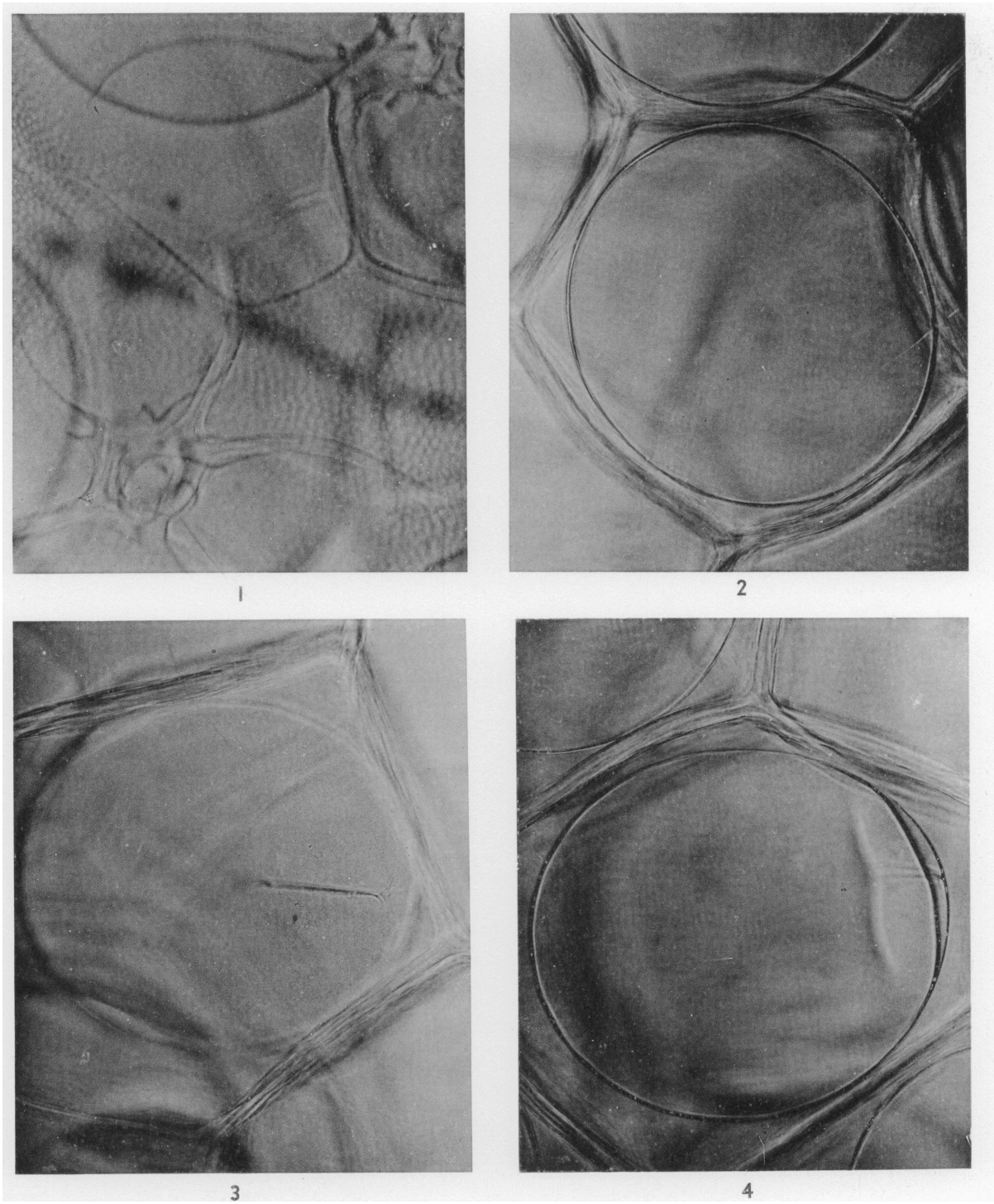
4

1. Section through ovary of *Antennarius*, showing similarity to ovary of *Histrio*. This ovary was filled mainly with resorbing ova. × 85

2. Detail of epithelium lining the lumen of *Antennarius* ovary, showing positive paraldehyde-fuchsin staining of proximal and distal parts of columnar cells. × 1100

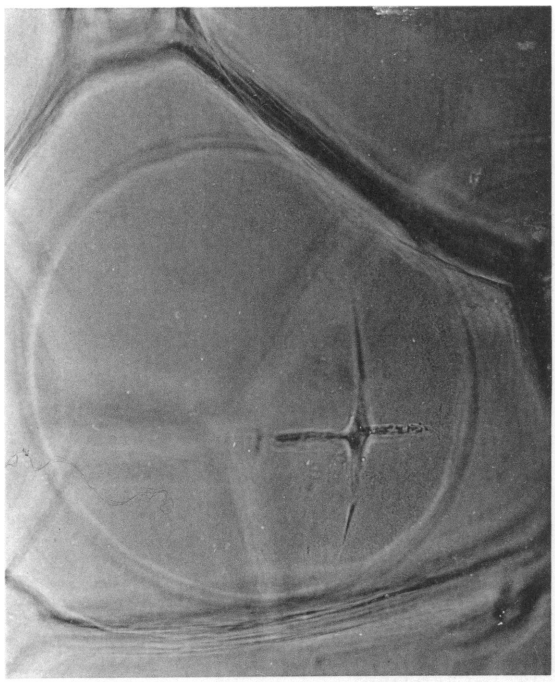
3. Section through ovary of *Ogcocephalus*, showing similarity to ovaries of *Histrio* and *Antennarius*, with ovigerous tissue on one side of lumen only

4. Low-power view of *Ogcocephalus* ovary, showing large blood vessels and sinuses of inner wall



1. View through gelatinous egg raft of *Antennarius* in which ova are embedded, showing membranes between eggs and surface view of one pore. Stippled appearance is characteristic of the outside envelope
2. Stage 1, the fertilized ovum
3. Stage 2, the first cleavage or two-celled ovum
4. Stage 2, the lateral view.

All × 115



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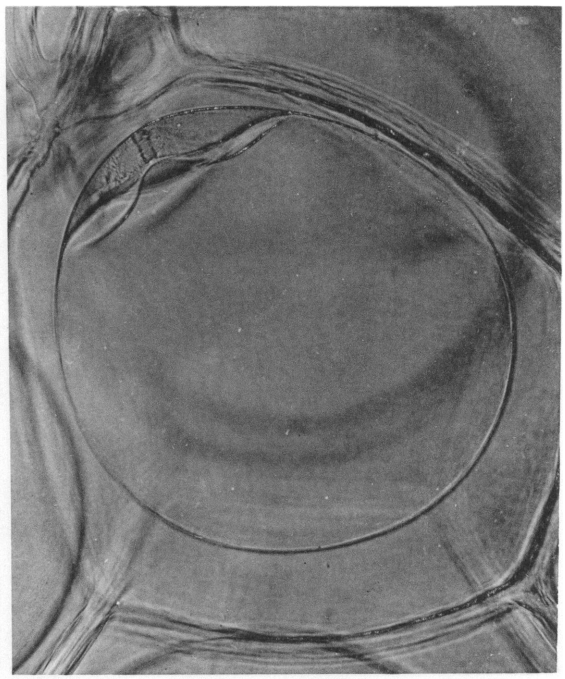

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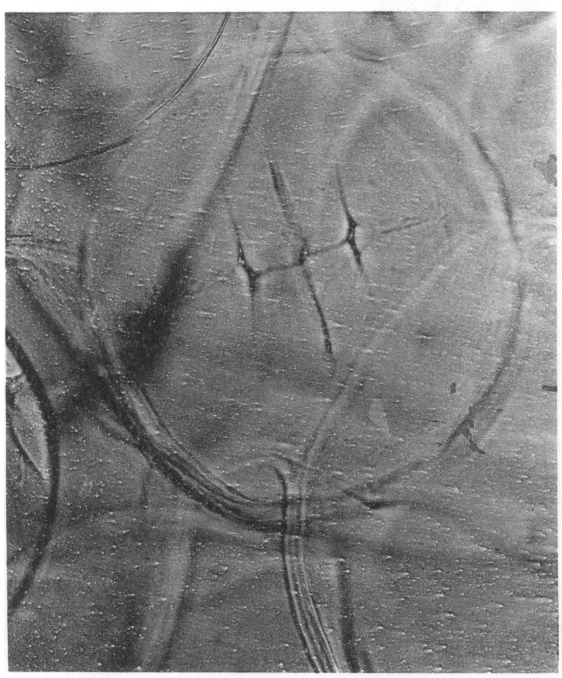

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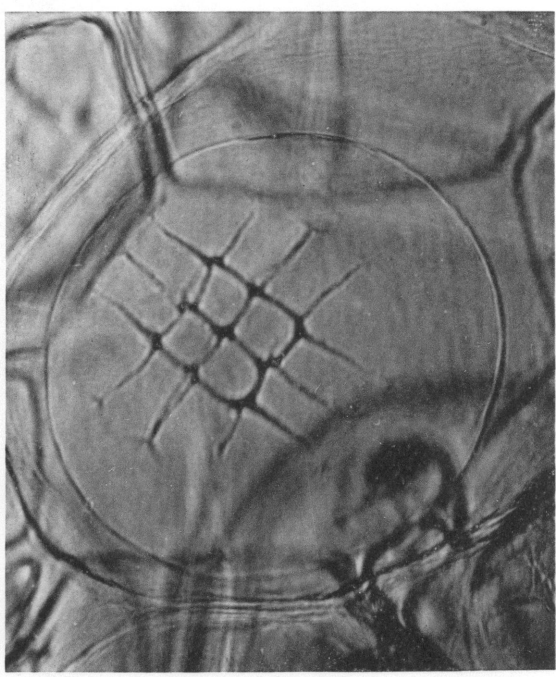
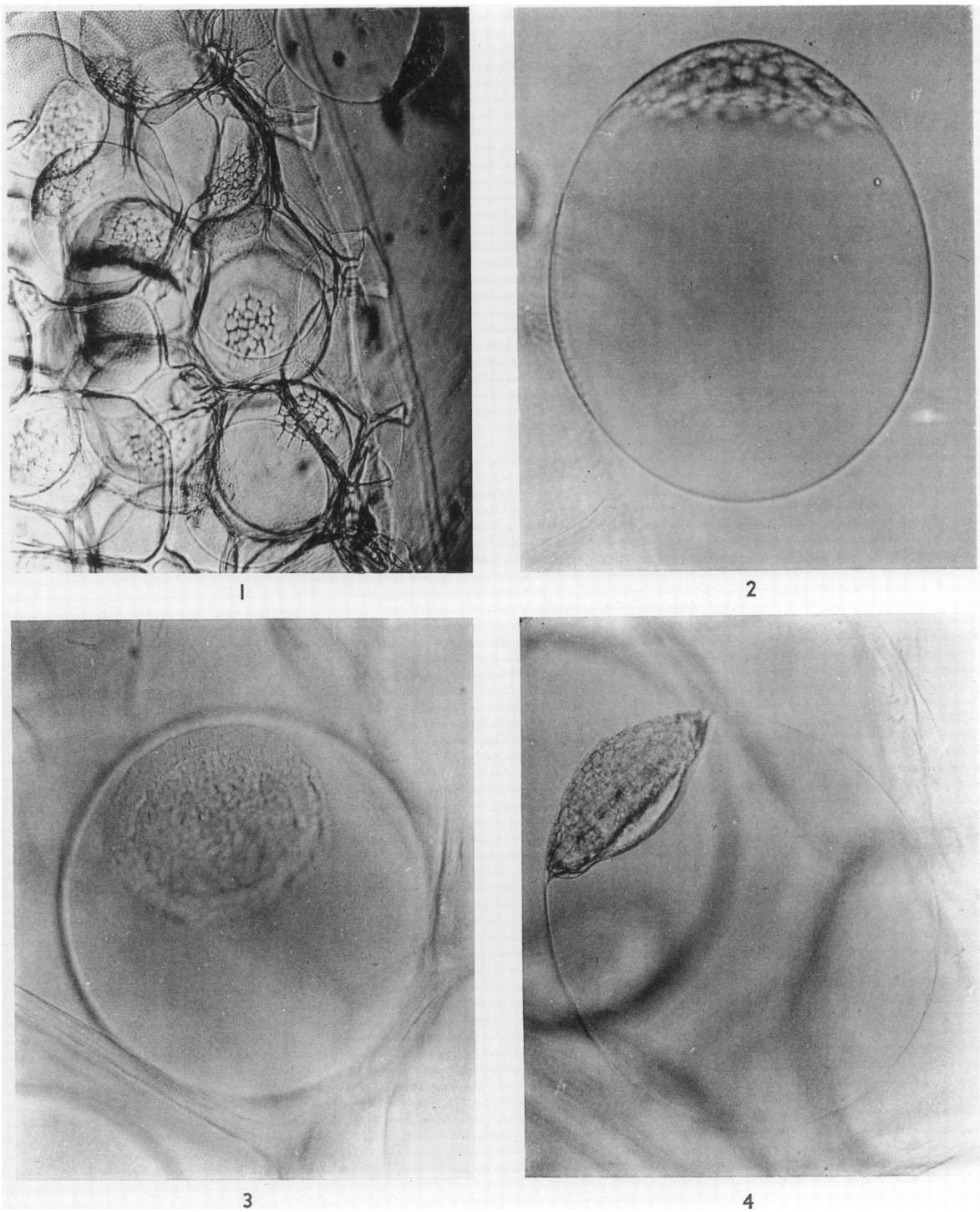


4

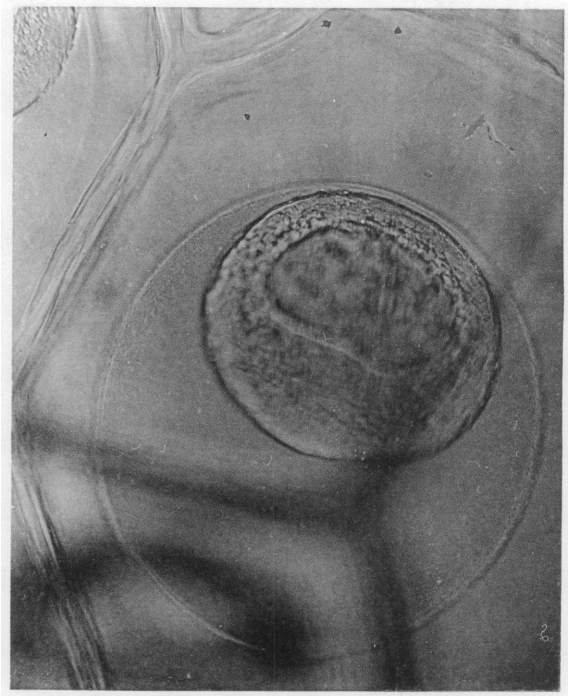
1. Second cleavage or four-celled stage. Stage 3
2. Lateral view of stage 3
3. Stage 4, third cleavage or eight-celled stage
4. Stage 5, fourth cleavage or 16-celled stage

All × 115



1. Stage 6, 32 cells. Magnification about × 30
2. Stage 7, lateral view, probably having 64 cells
3. Stage 9, late blastula or germ ring, showing thinning of center of blastodisc to form central periblast
4. Stage 9, lateral view showing beginning of a zone of junction and segmentation cavity

2, 3, 4 are × 115



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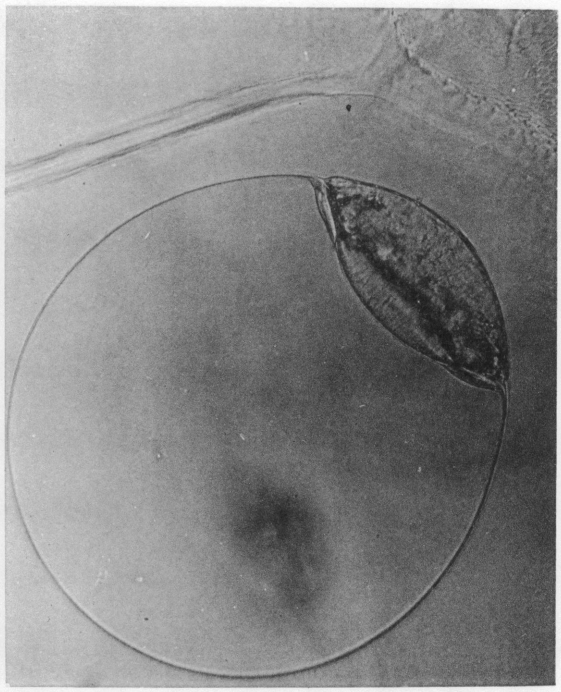

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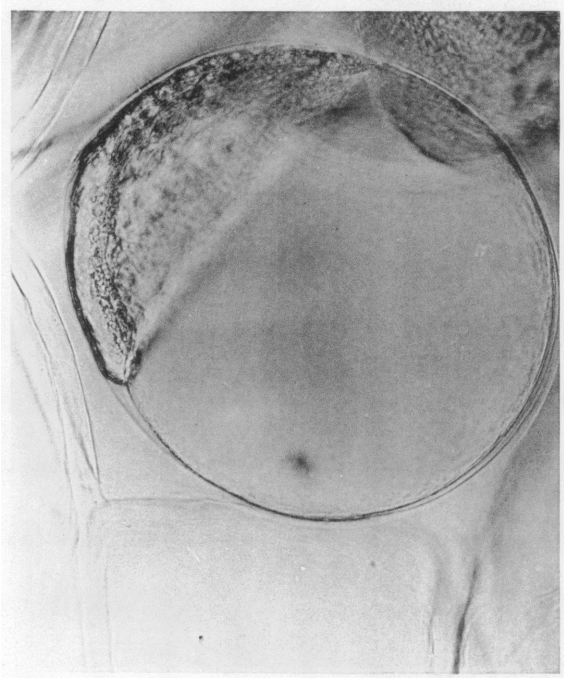

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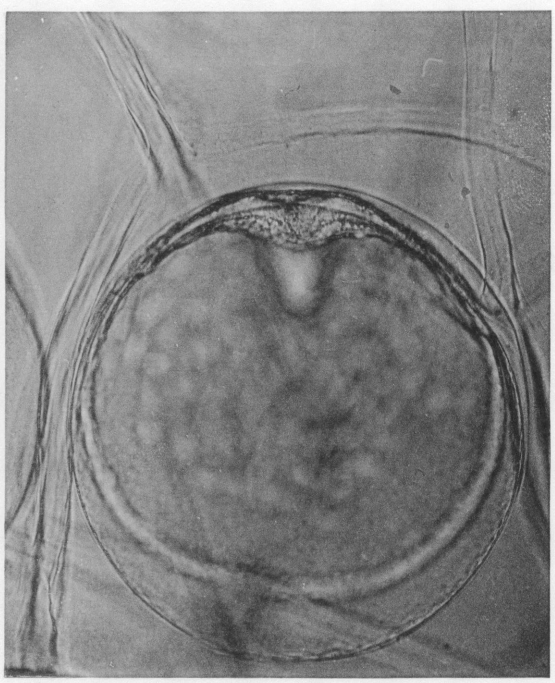
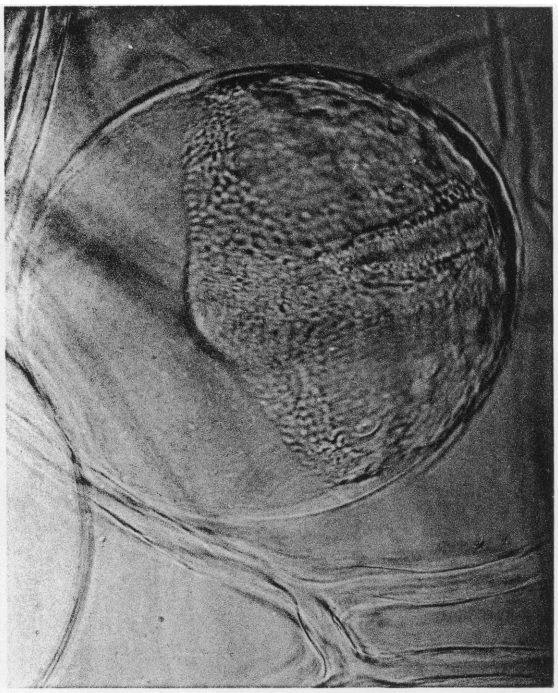


4

1. Stage 10, early gastrula, showing embryonic shield and establishment of anterior-posterior axis
2. Stage 10, lateral view, showing enlarged zone of junction and deepened segmentation cavity
3. Stage 11, showing neural keel, marginal periblast, and narrow perivitelline space
4. Stage 11, ventral view looking through the yolk, showing extent of periblast

All × 115



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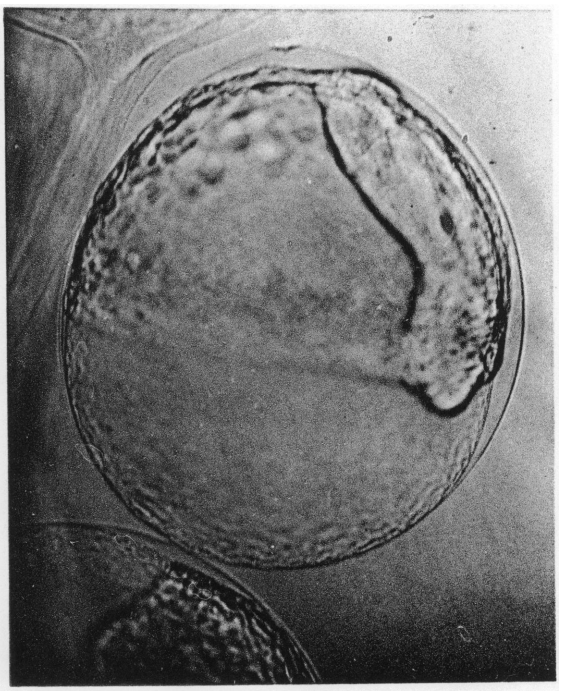

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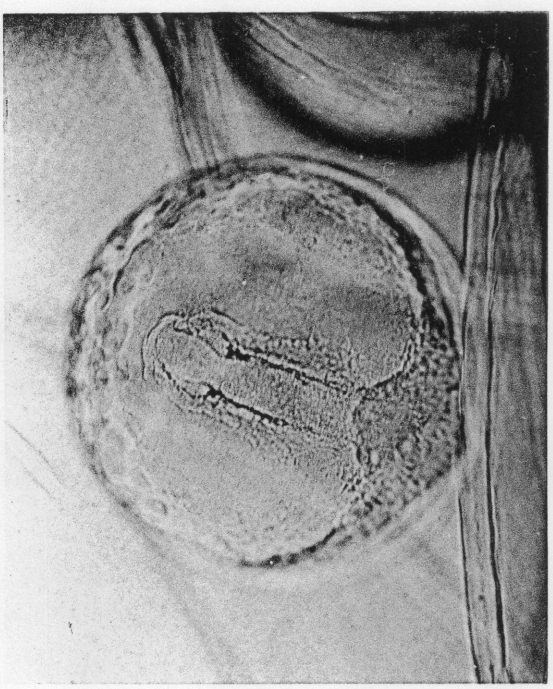

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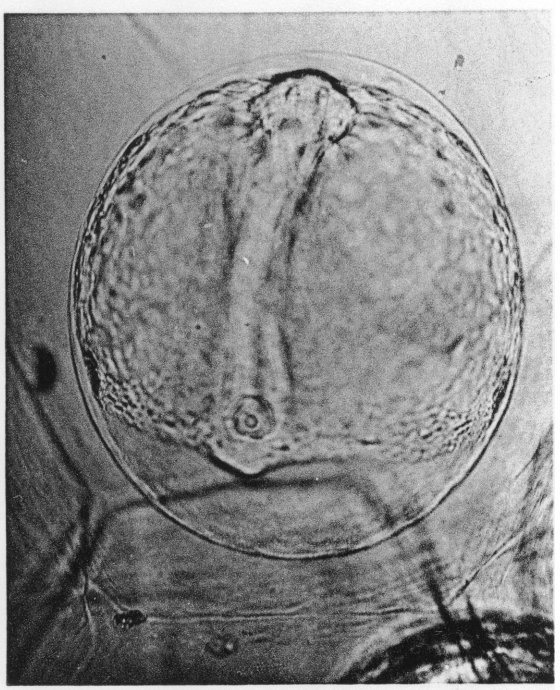
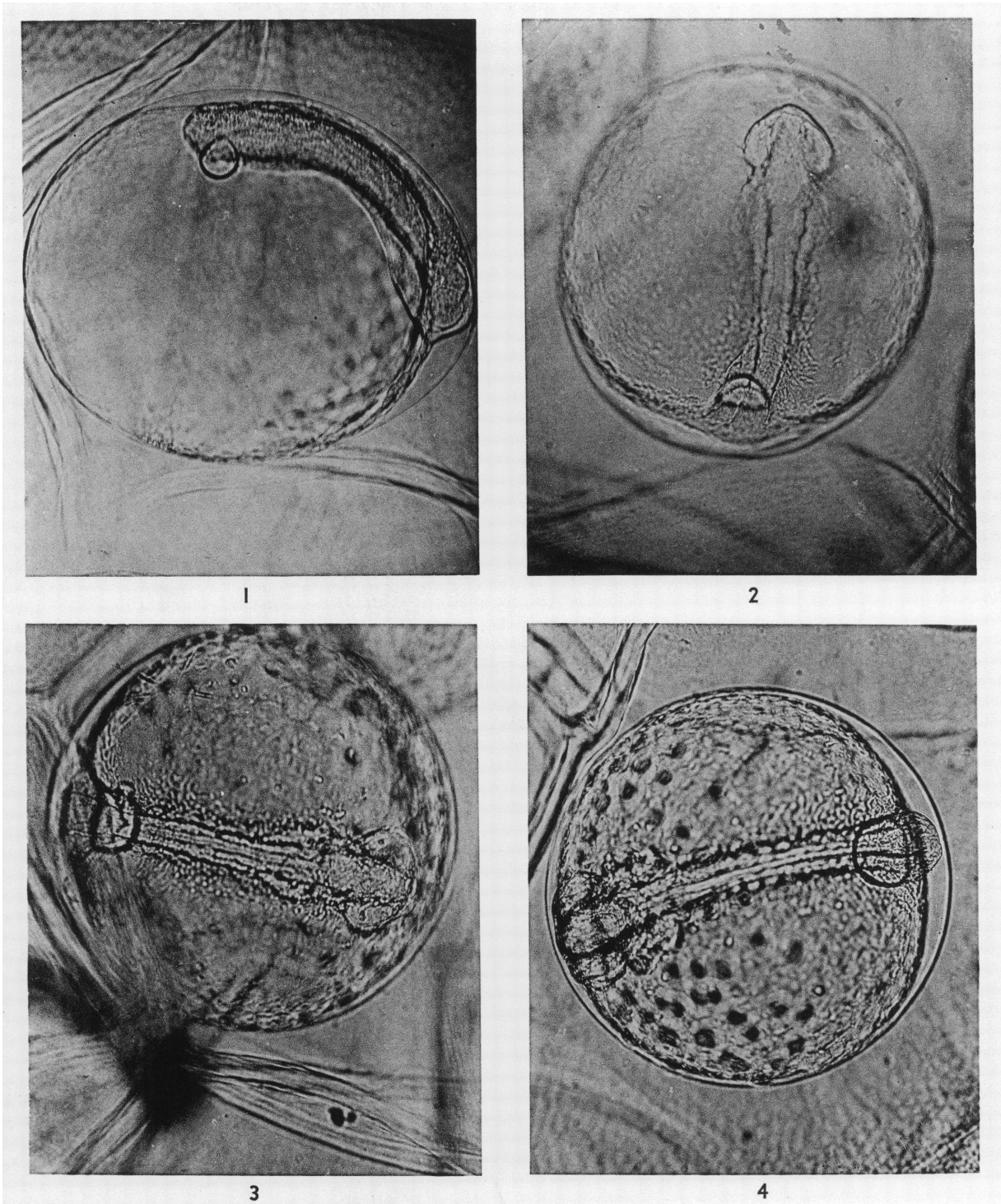


4

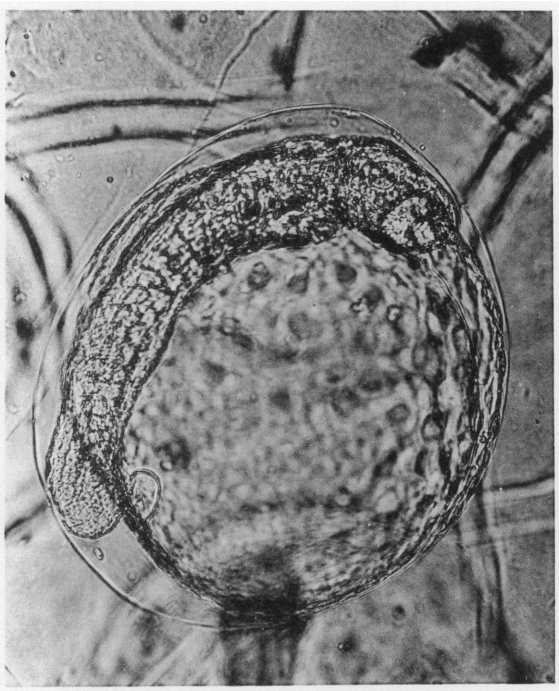
1. Late stage 11, showing increase in area of marginal periblast
2. Stage 12, lateral view, showing perivitelline space and embryo raised above surface of yolk
3. Stage 13, showing formation of optic vesicles and differentiation of mesoderm at the sides of neural keel
4. Stage 14, showing Kupfer's vesicle and further increase in area of marginal periblast

All × 115



1. Stage 14, showing formation of lens and separation of main divisions of brain
2. Stage 15, showing blastopore not yet closed and the beginning of somite formation
3. Late stage 15, somites, auditory placodes, and further differentiation of optic vesicles
4. Stage 16, showing additional somites, increased differentiation of auditory vesicles, and lateral outpushing of eyes

All × 115



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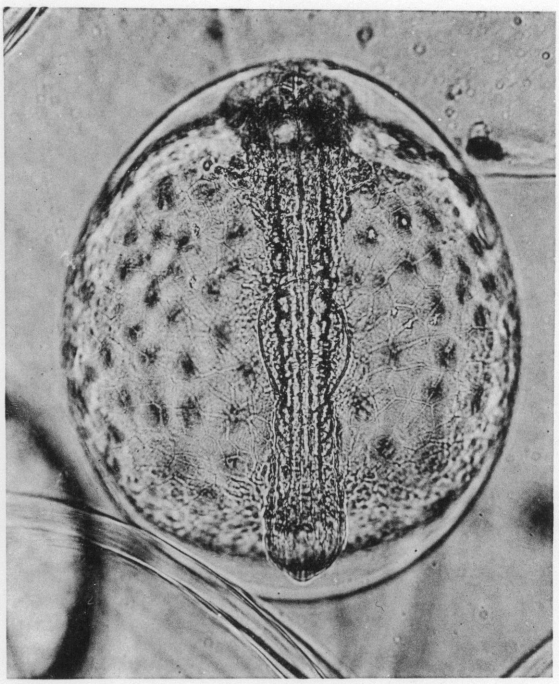

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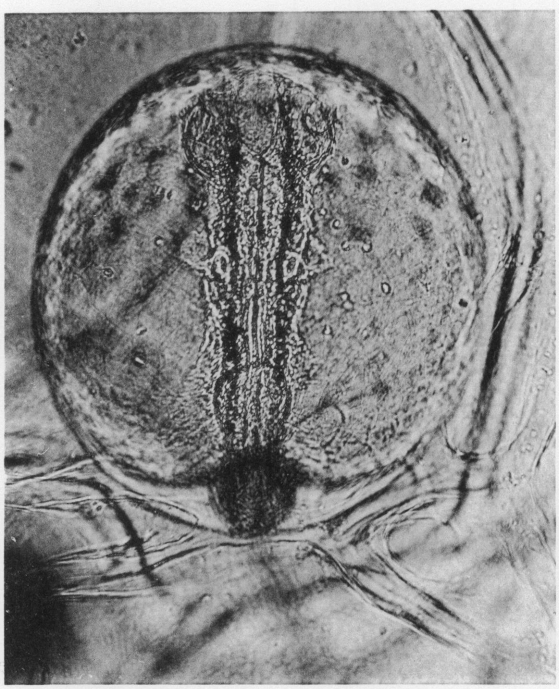

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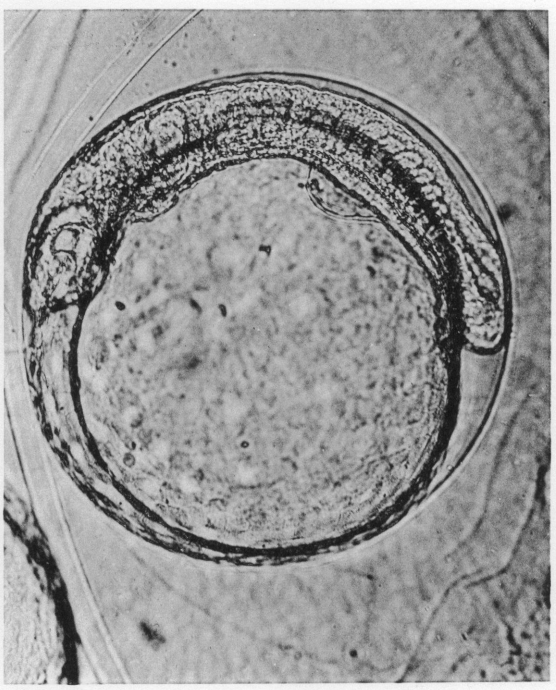
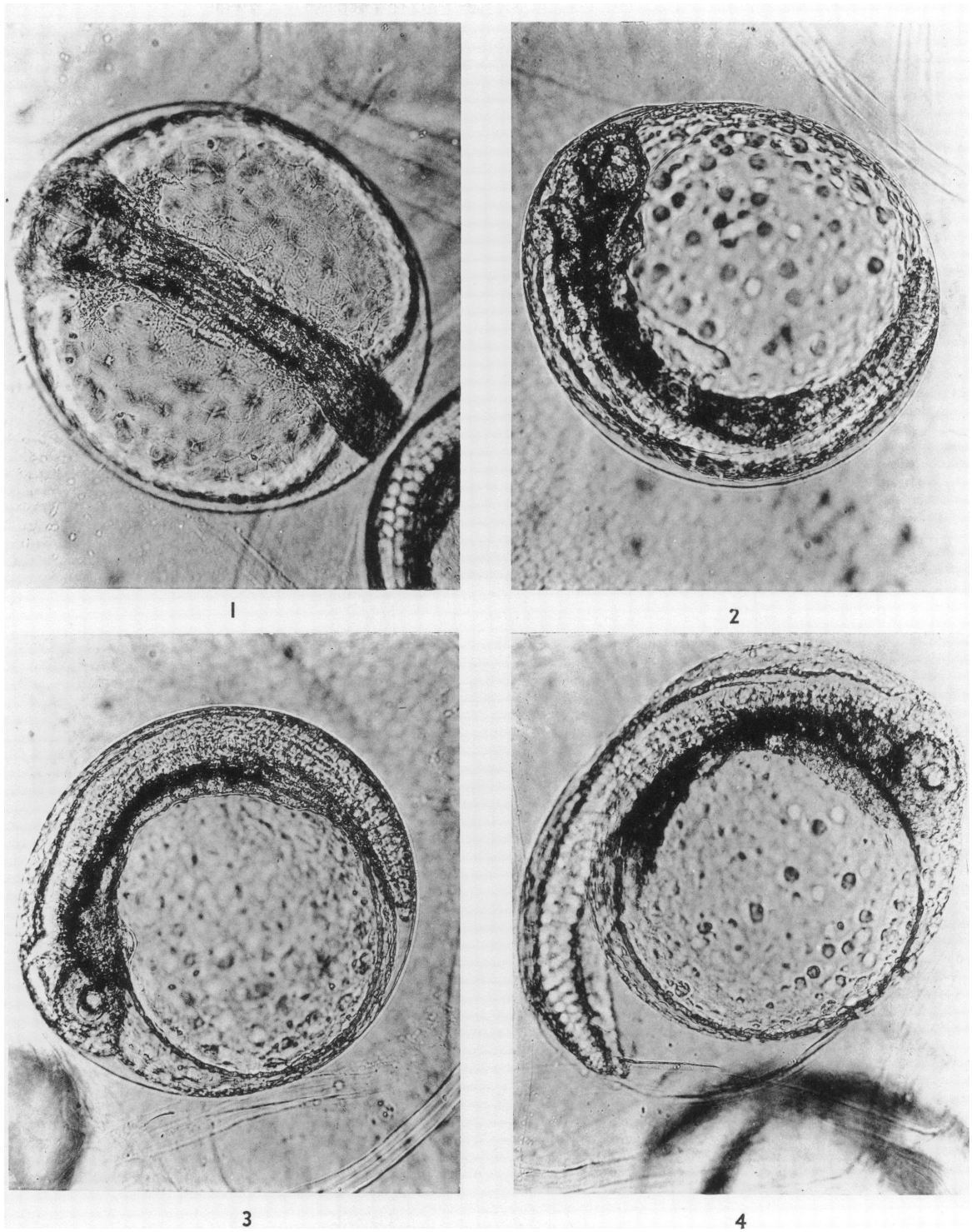


4

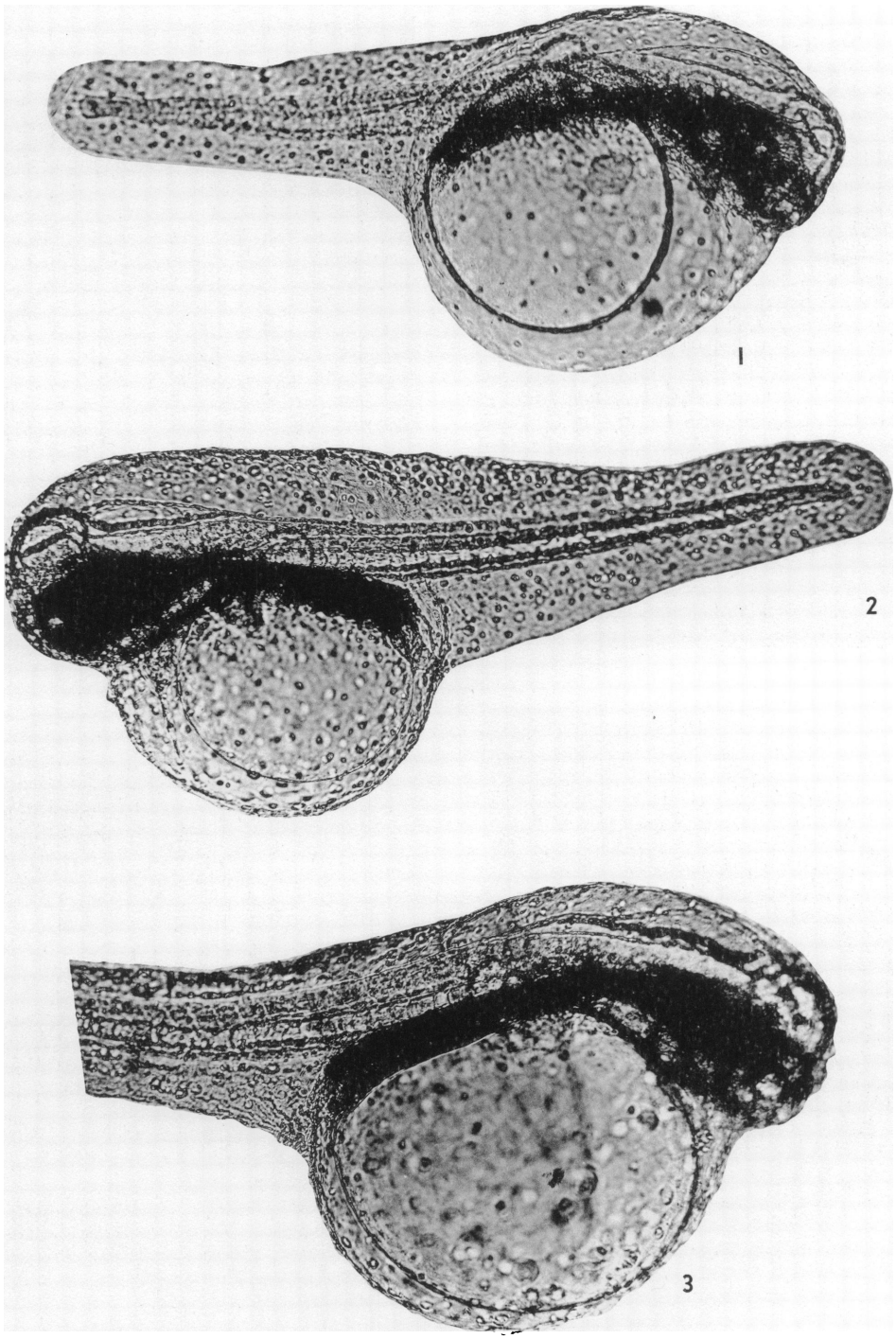
1. Stage 16, lateral view, showing somites, free tail bud, otic capsule, and specialized cells of outside ectoderm
2. Stage 16, dorsal view, showing aberrant placement of Kupfer's vesicle and lateral outpushing of gill slit
3. Late stage 16, ventral view, showing gill slit
4. Late stage 16, lateral view, showing pectoral fin bud, metameres, and end of notochord

All × 115



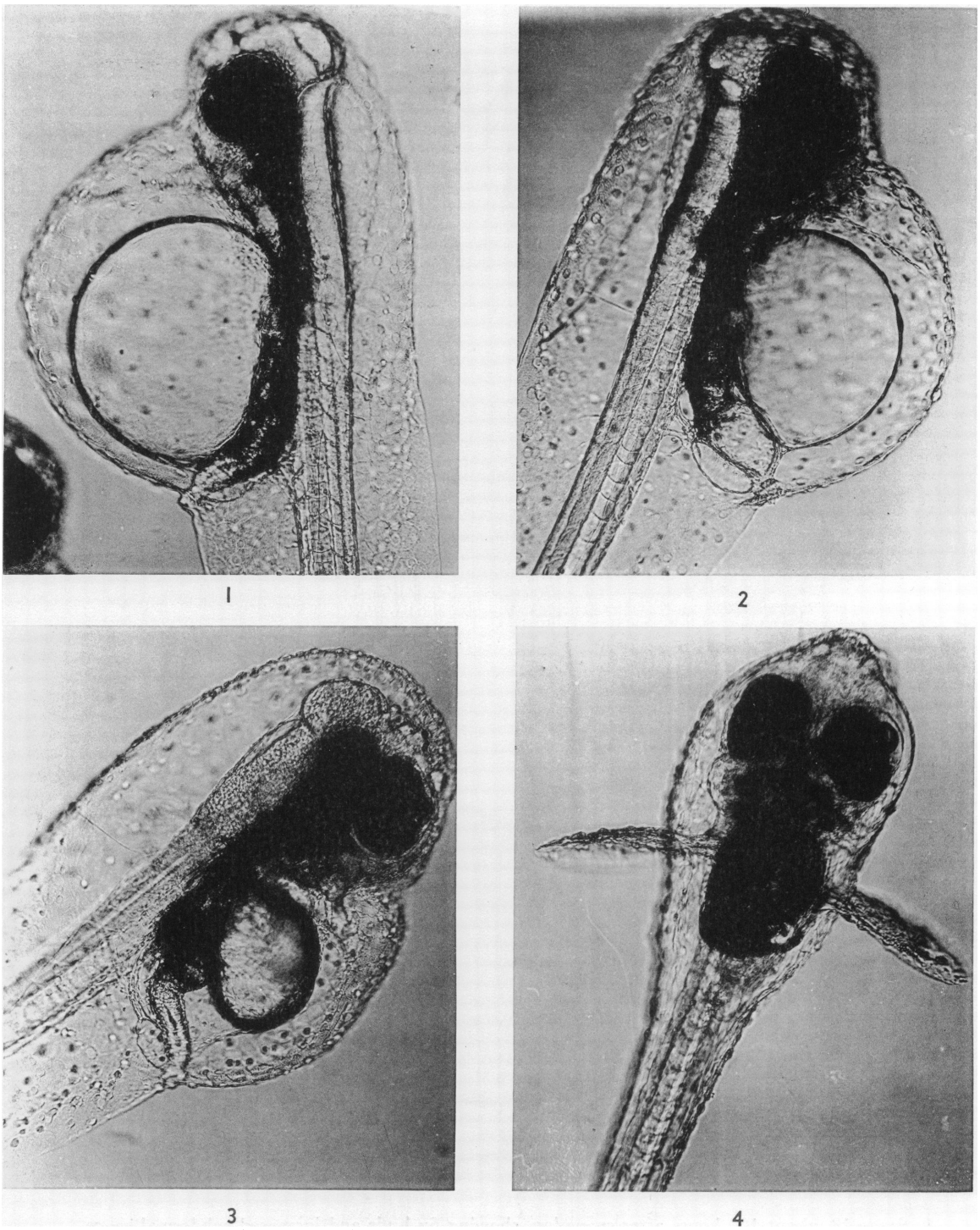
1. Stage 17, ventral view, showing lateral extension of gill slit
2. Stage 17, lateral view, showing slight flexion of head, brain differentiation, and region of the heart
3. Stage 17, lateral view, showing brain differentiation, heart, and metameres of tail
4. Late stage 17, lateral view of larva about to hatch, with tail free of yolk sac

All × 115



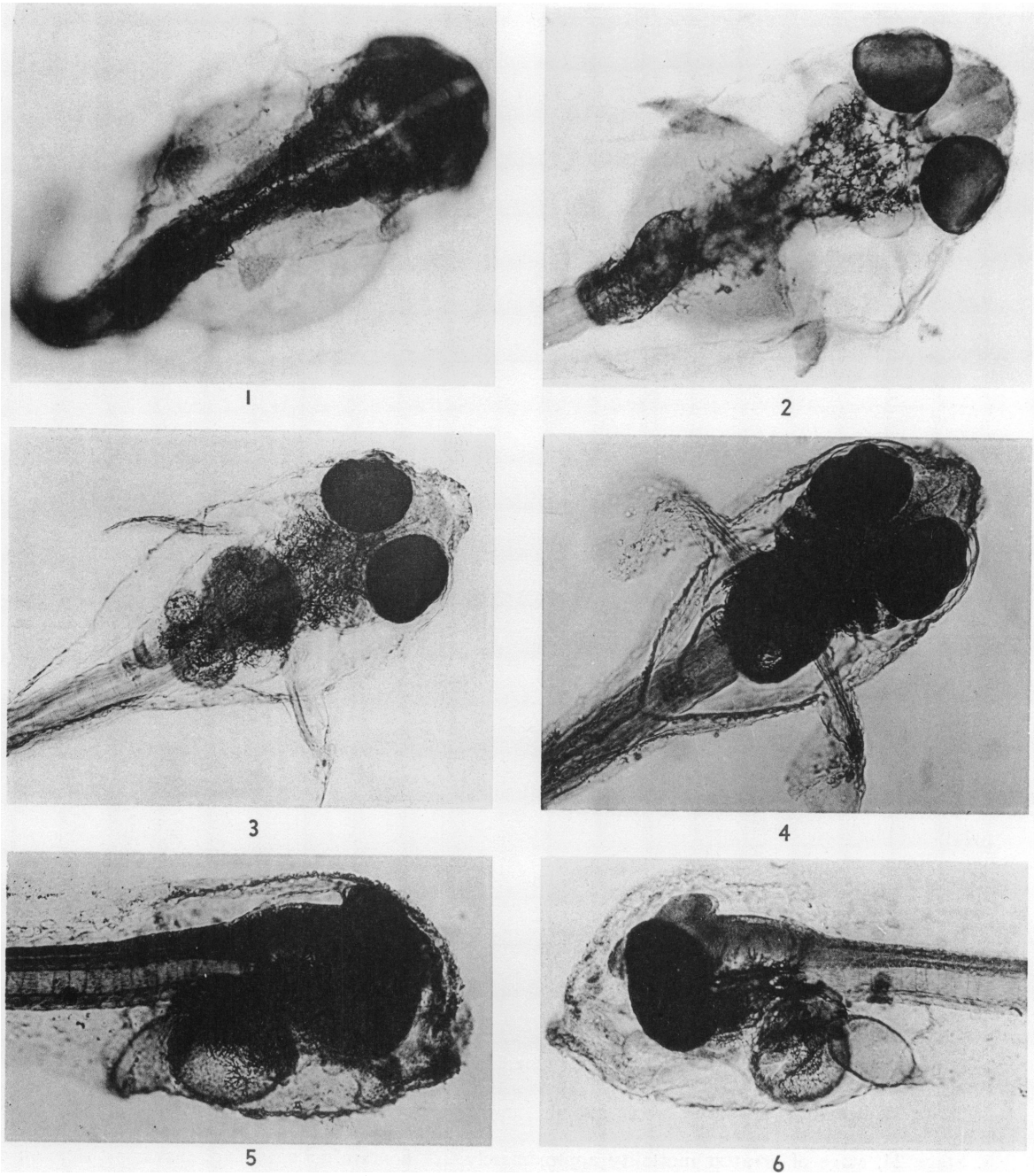
1. Stgae 18, about 100 hours after spawning, showing fin buds and increased subdermal space
2. Early stage 18 about 77 hours after spawning, showing thin roof of fourth ventricle and nerve cord and notochord
3. Late stage 18, showing the same features and the aggregations of cells of the outside ectoderm. (Enlargement too great for entire embryo to be included)

All × 115

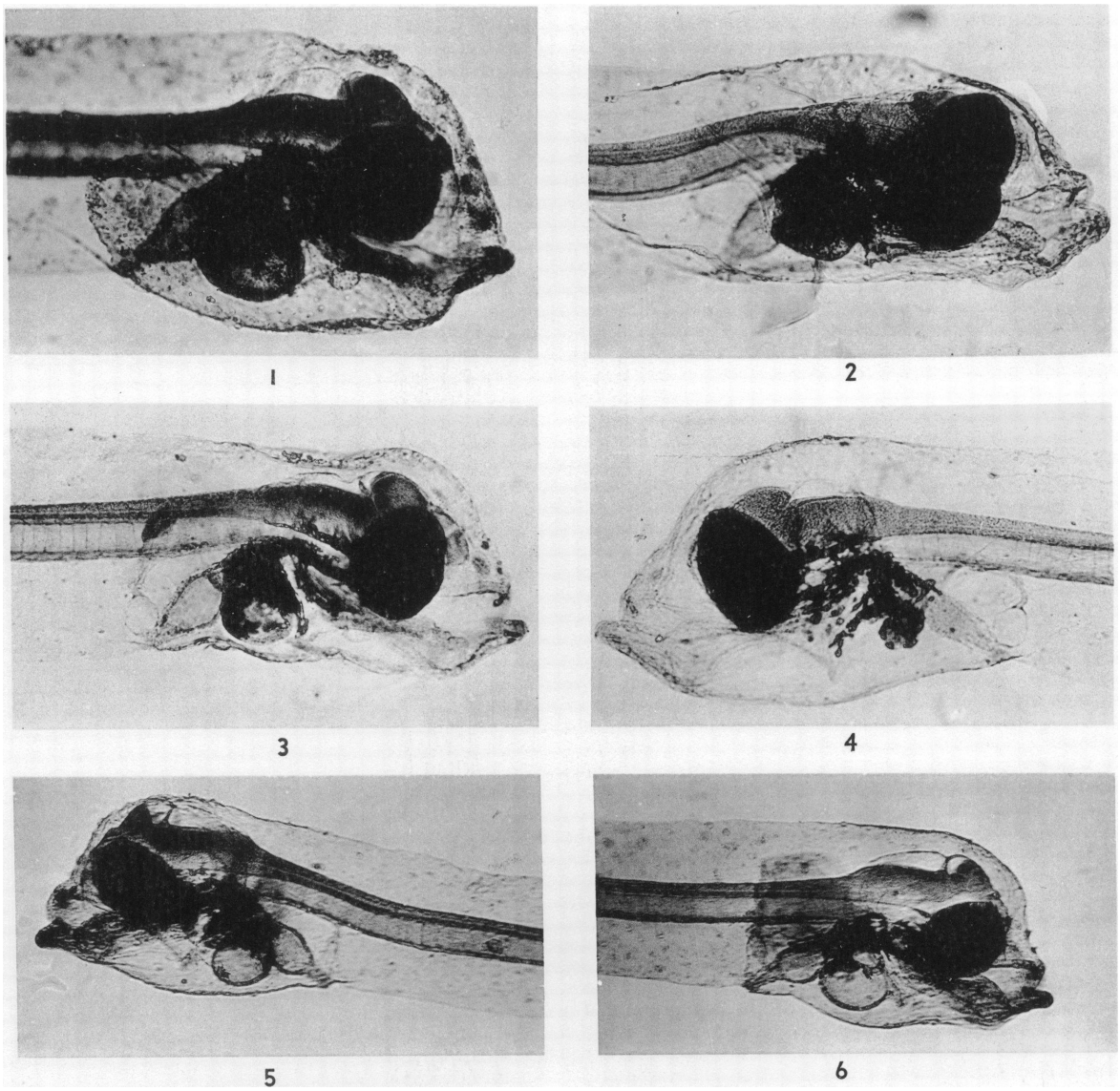


1. Stage 19 approximately 114 hours after spawning, showing heart, myomeres, and urinary vesicle
2. Stage 19 approximately 117 hours after spawning, showing reduction in yolk sac, enlarged urinary bladder and pronephric ducts, and slightly distended intestine
3. Stage 20 approximately 125 hours after spawning, showing further reduction in size of yolk sac. A thin membrane representing peritoneum is visible between anus and yolk sac
4. Stage 21 about 148 hours after spawning, showing forward position of eyes, lens, and cornea and extent of pectoral fins

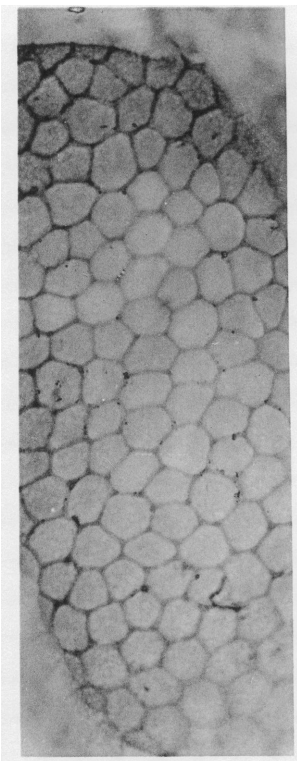
All × 115

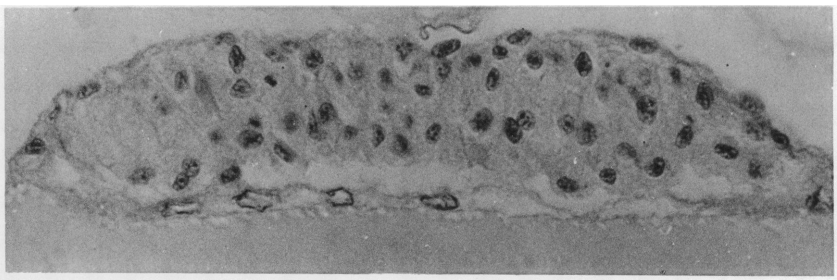


1. Stage 16, hematoxylin-stained whole mount, showing pectoral fin buds
2. Stage 19, alizarin-stained whole mount, showing development of pectoral fins, characteristic pigmentation, and otic capsules
3. Stage 21, alizarin-stained whole mount, showing cartilaginous elements of pectoral fin
4. Stage 22, dorsal view of alizarin-stained whole mount, showing cartilage of basal fin bone and distal membrane where fin rays will ultimately appear
5. Stage 22 approximately 172 hours after spawning, lateral view of hematoxylin-stained whole mount, showing forward rotation of eyes, yolk sac, and distended gut
6. Late stage 22, showing development of jaws, pigmentation, and distended gut. Nasal placodes also visible



1. Stage 23 approximately 200 hours after spawning, hematoxylin-stained whole mount, showing jaw muscles and heart
2. Stage 23, hematoxylin-stained whole mount, showing abnormality of curved notochord and decreased affinity for the stain
3. Stage 24, stage of greatest mortality approximately 225 hours after spawning, showing distension of gut with gas
4. Later stage 24, approximately 250 hours after spawning, showing clumped condition of melanin
5. *Histrio*, hematoxylin-stained whole mount in stage 23, showing similar gas distension of gut, eight days after spawning
6. *Histrio*, stage 24 or possibly later, showing anlage of first and second dorsal spines and of pineal organ, nine days after spawning





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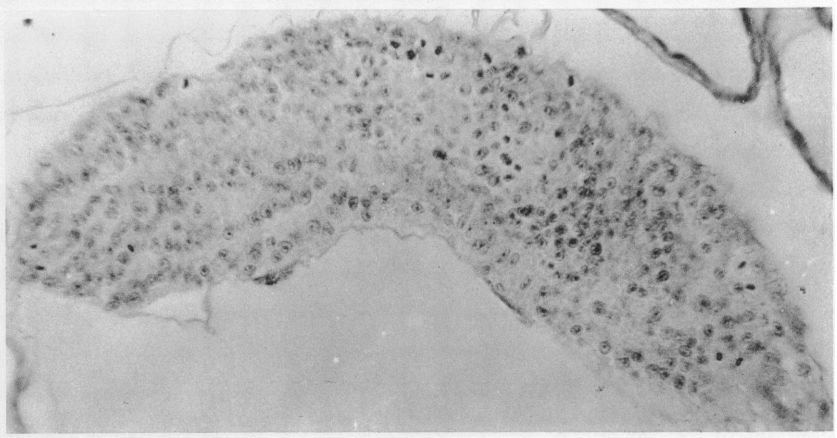

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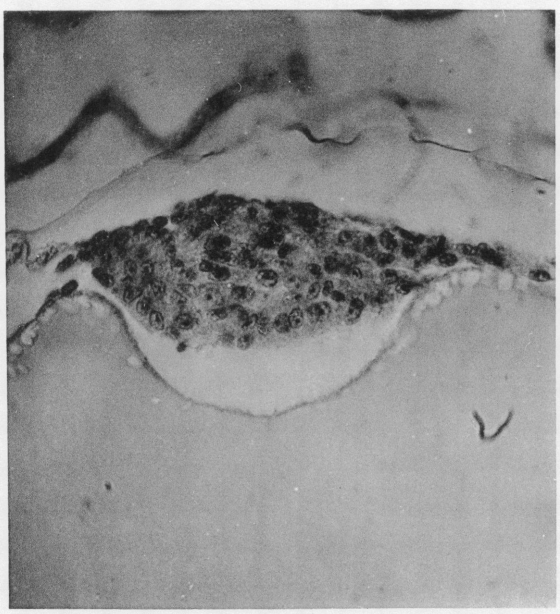
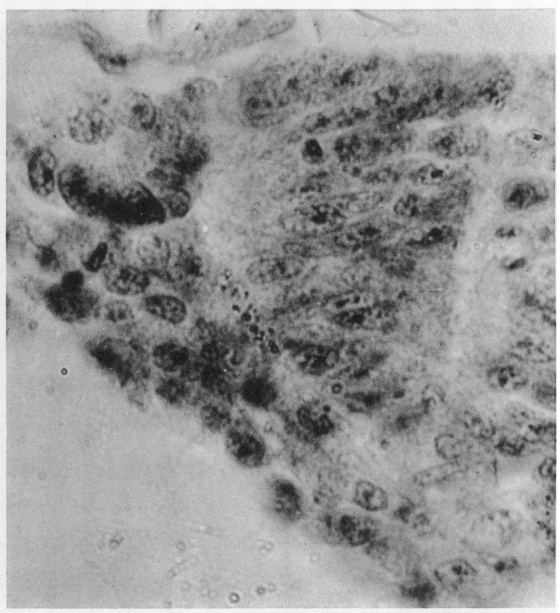
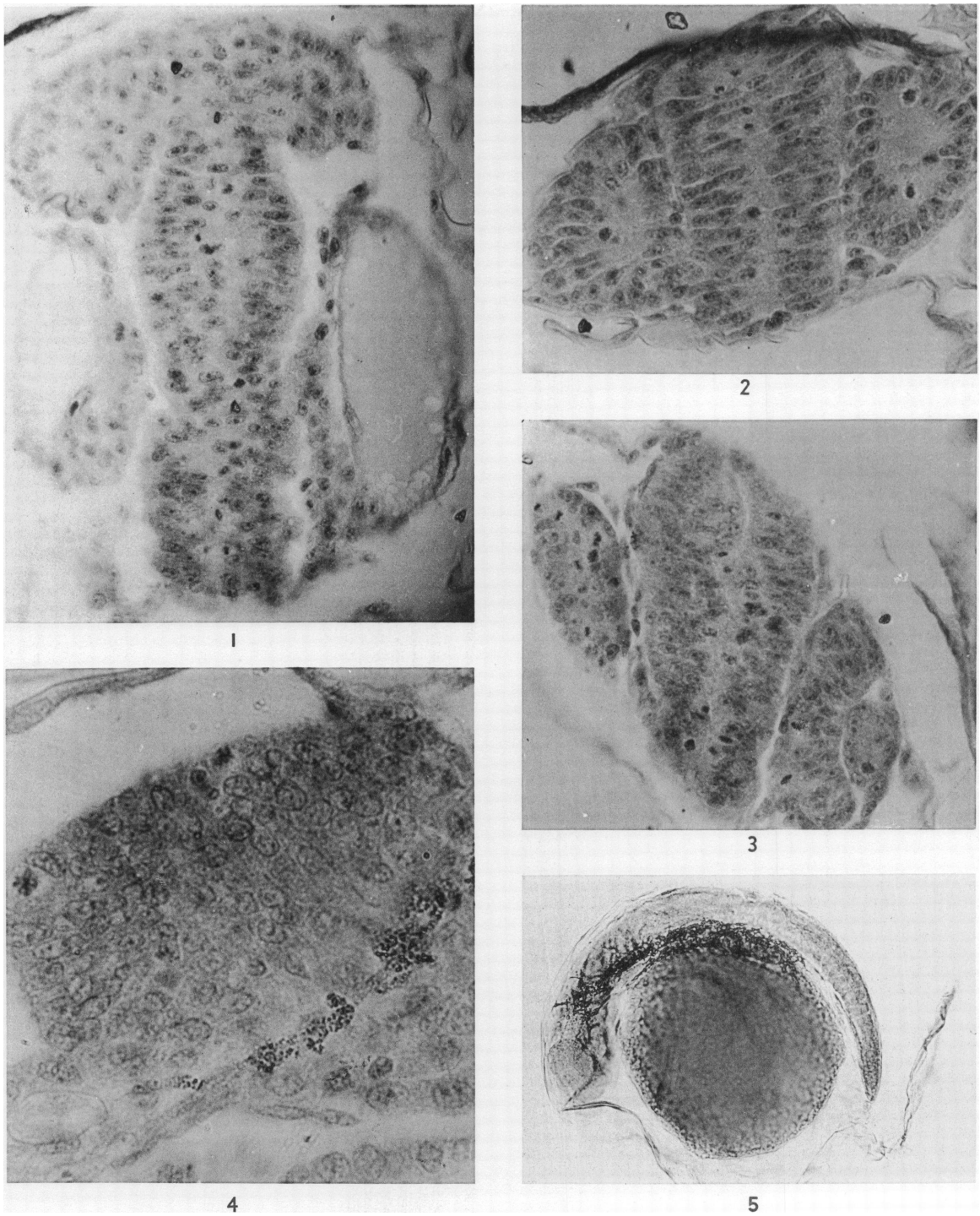




4

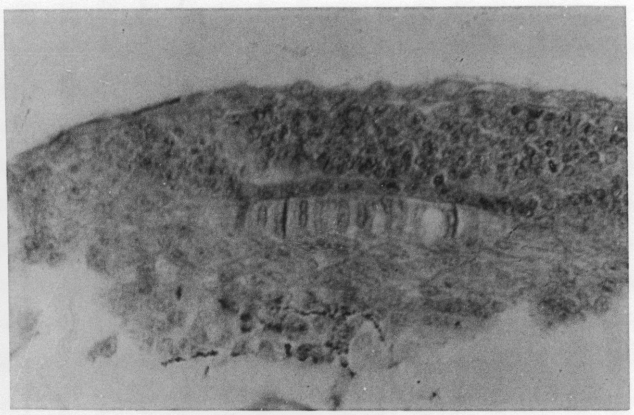
5

1. Horizontal section through gelatinous envelope which surrounds egg raft
2. Stage 8, section showing cells of germinal disc, segmentation cavity, and syncytium of cells which is the central periblast forming floor of cavity
3. Stage 11, sagittal section, showing ventral endoderm with thicker overlying layer consisting of neural plate and presumptive notochord
4. Stage 14, section through Kupfer's vesicle
5. Stage 14, section through anterior portion of neural plate, showing beginning tubulation, separation from overlying ectoderm, and melanin granules at ventrolateral aspect of neural plate



1. Stage 13, horizontal section, showing untubulated condition of neural plate and optic vesicles at this time
2. Stage 13, transverse section through optic vesicles, showing neural crest tissue
3. Stage 15, oblique section, showing development of lens and invagination of optic cup
4. Stage 15, section through posterior end of optic vesicle, showing position of melanophores
5. Stage 15, alizarin-stained whole mount, showing distribution of melanophores

1



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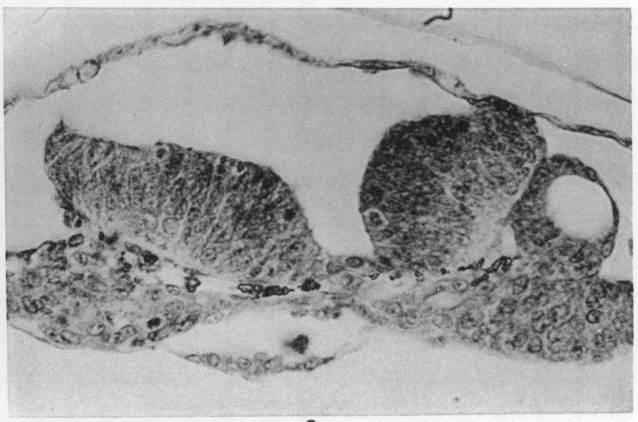

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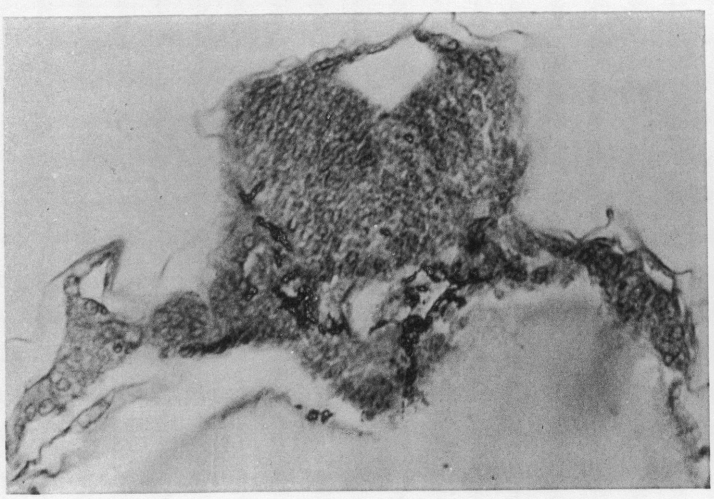

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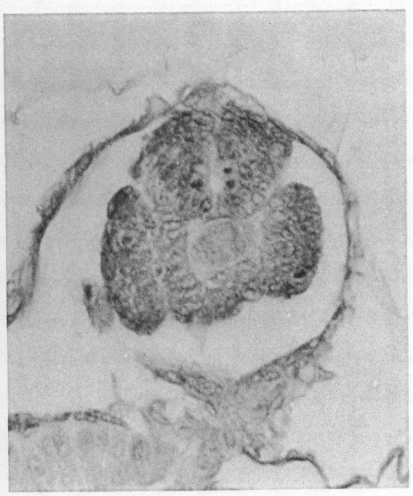

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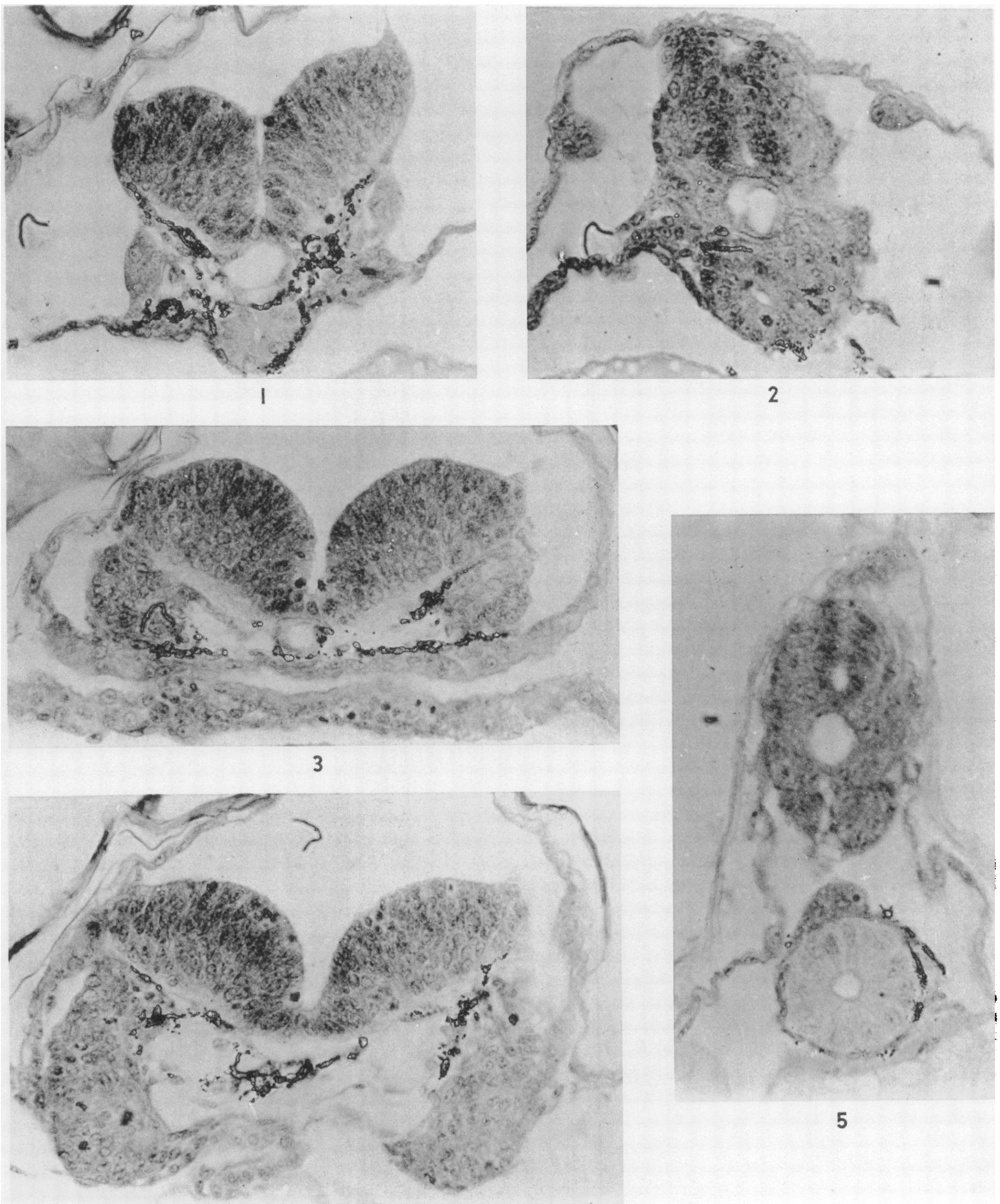
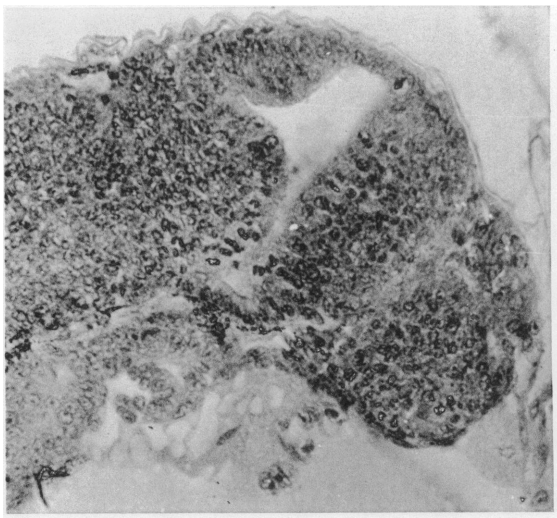
1. Stage 15, horizontal section through posterior region of gut, showing disposition of melanophores
2. Stage 15, sagittal section showing notochord and dorsal rod of cells beneath overlying neural tube tissues
3. Stage 15, oblique section showing lumen of auditory vesicle
4. Stage 15, transverse section just posterior to auditory vesicle, showing disposition of pigment, thin roof of myelencephalon, and somitic mesoderm
5. Stage 15, section through trunk region, showing developing cranial nerves



1. Stage 16, transverse section showing developing cranial nerves
2. Stage 16, transverse section showing developing spinal nerve and anlagen of sense organs of lateral line
3. Stage 16, transverse section through gill slit
4. Stage 16, transverse section through heart, showing opening into yolk-sac sinus
5. Stage 16, transverse section showing liver anlage



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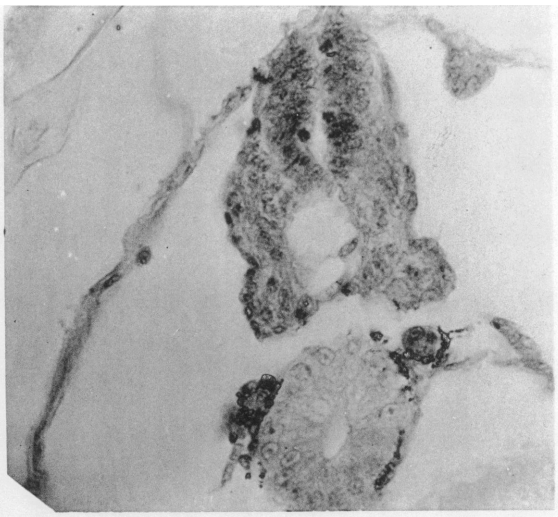

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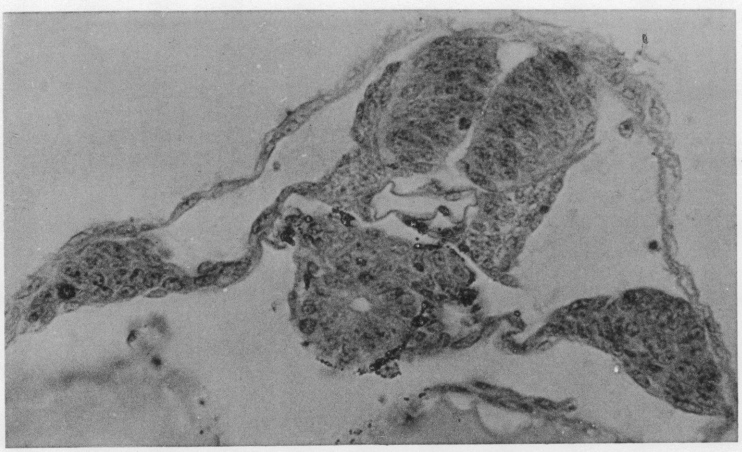

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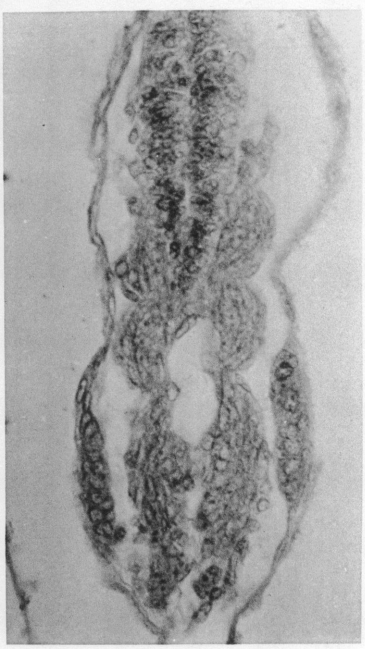

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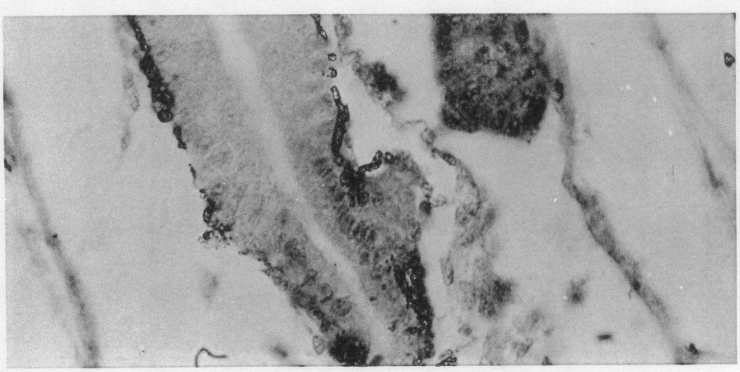

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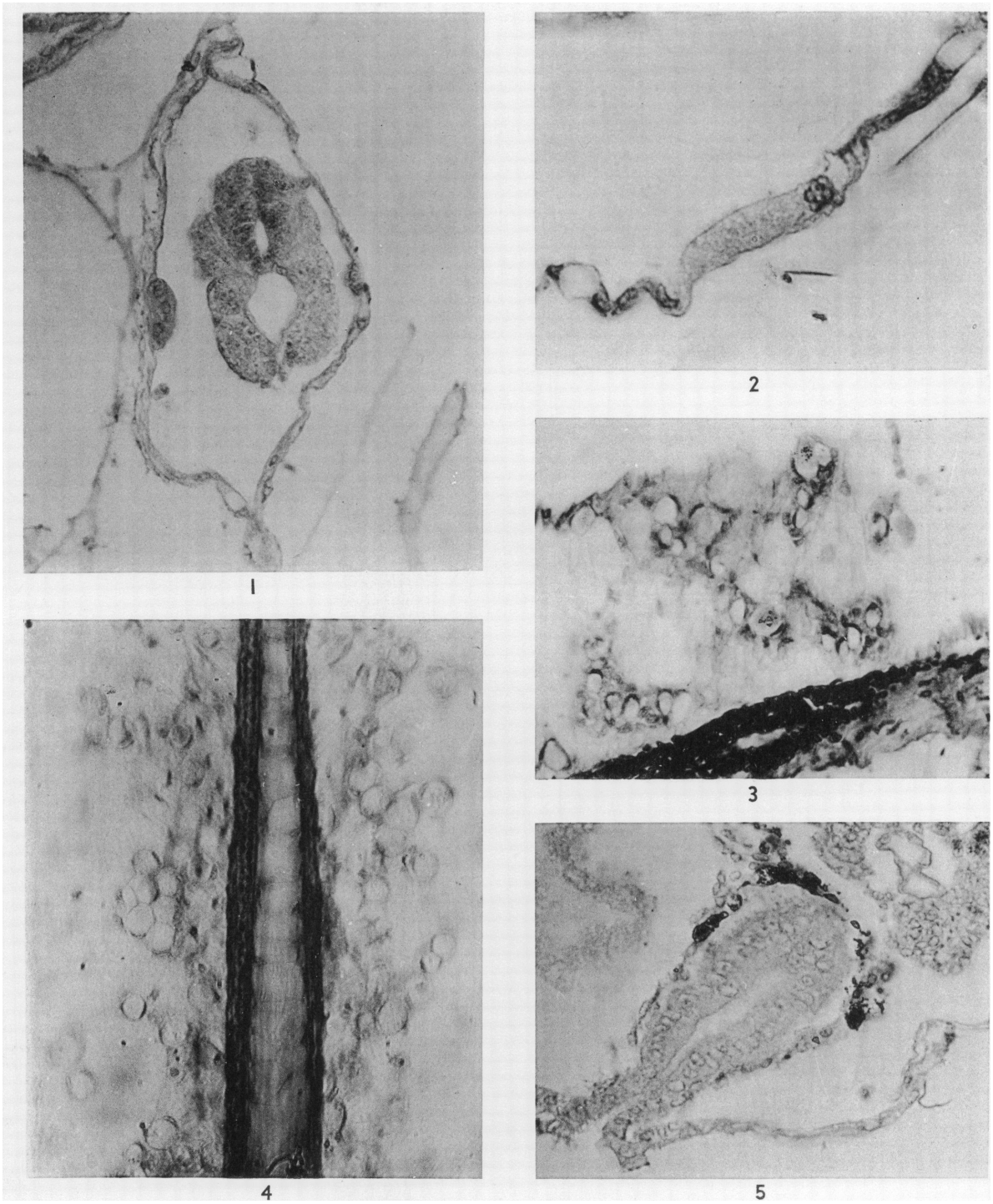
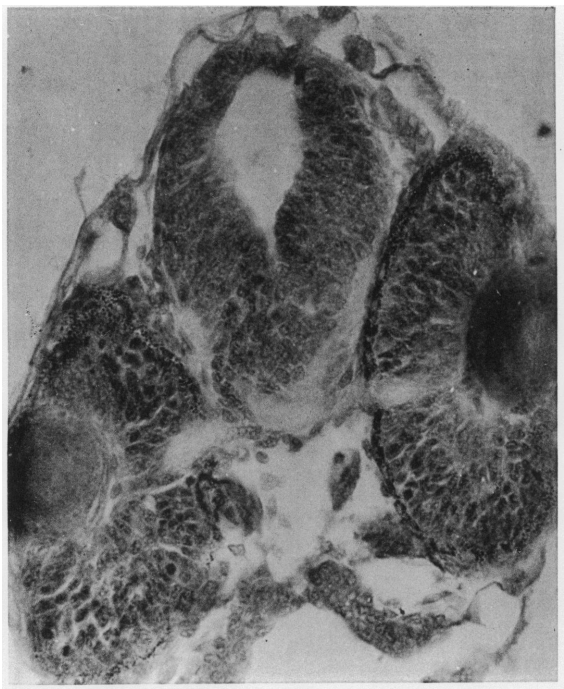
1. Stage 17, sagittal section showing third ventricle and developing heart
2. Stage 17, transverse section showing pronephric ducts
3. Stage 17, transverse section showing pectoral fin buds
4. Stage 17, sagittal section showing liver outpocketing from gut
5. Stage 17, oblique section showing anlagen of sense organs of lateral line



1. Stage 18, transverse section through caudal region, showing lateral-line sense organ, body musculature close to notochord, and large area of subdermal space
2. Stage 18, transverse section through outside ectoderm, showing cell types
3. Stage 18, oblique section through dorsal fin fold, showing arrangement of cells
4. Stage 18, portion of caudal area of *Histrio* taken from hematoxylin-stained whole mount, showing similar structure of outside membrane
5. Stage 18, oblique section showing open anus



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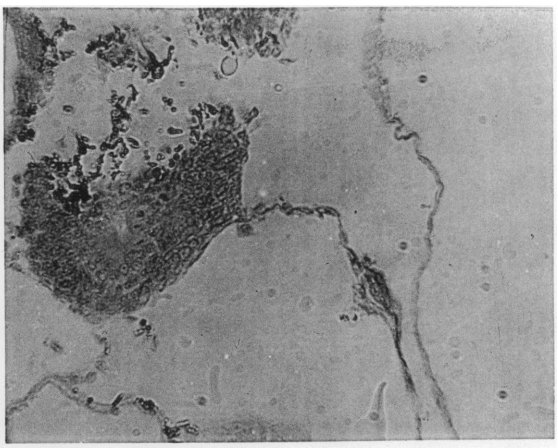

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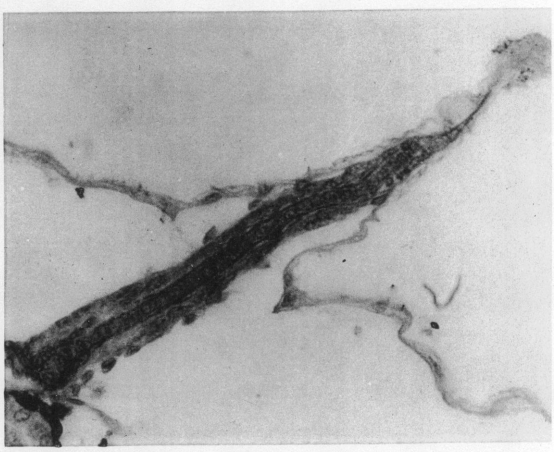

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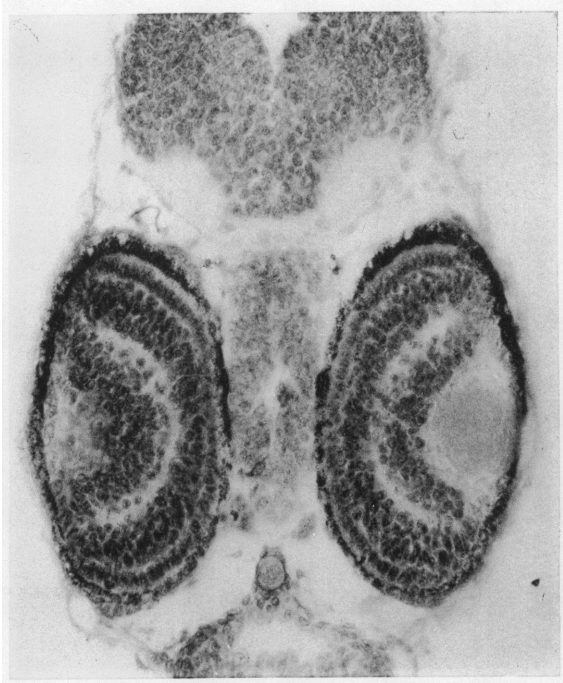

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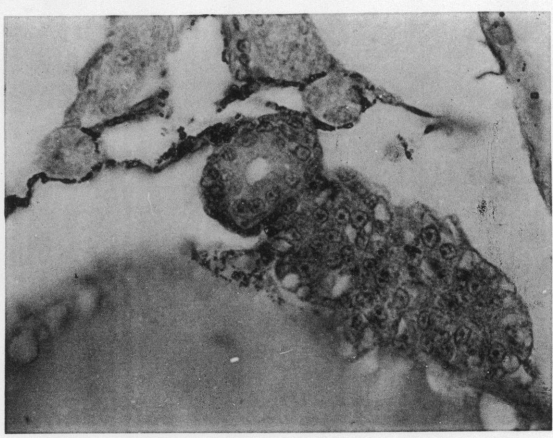

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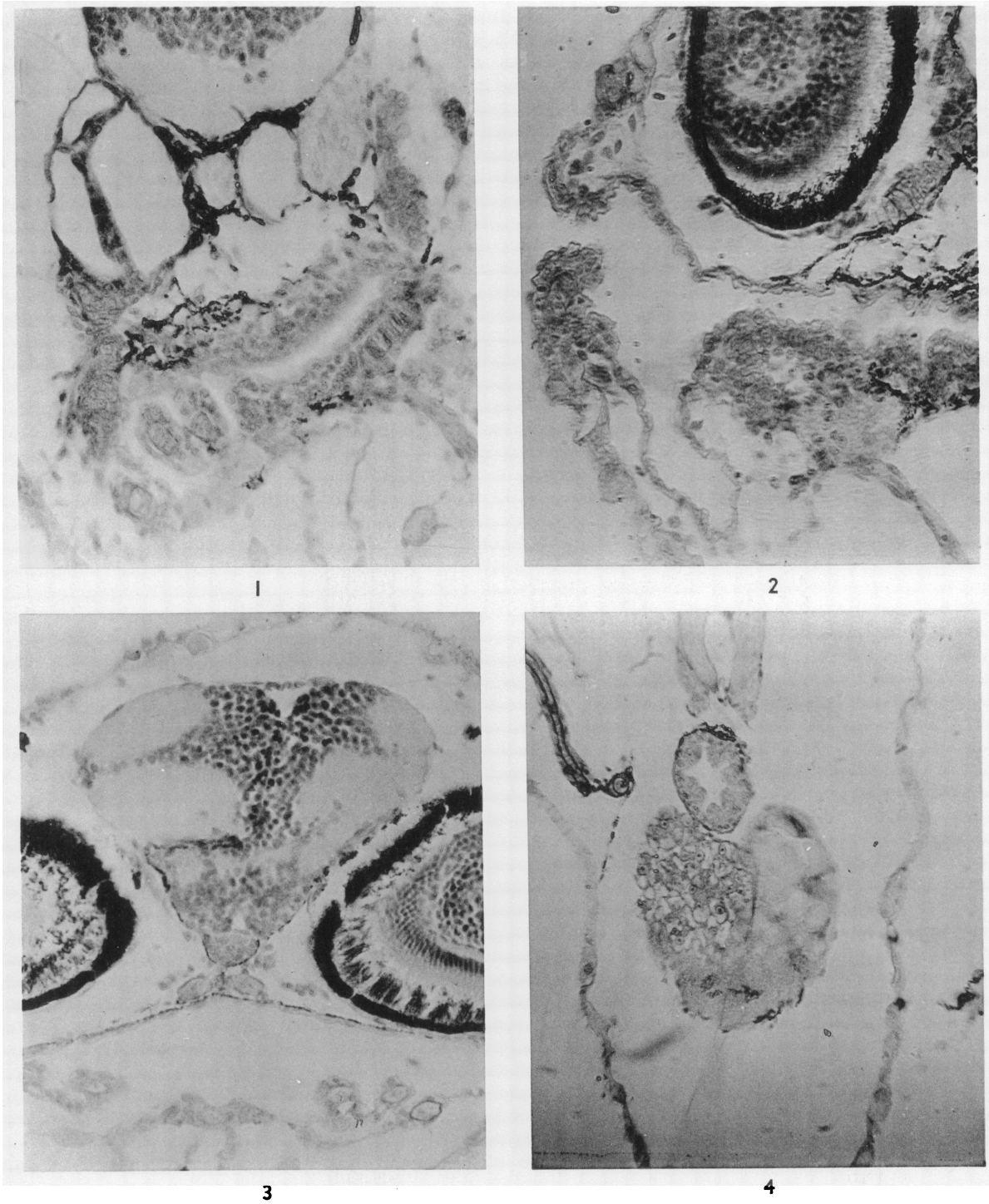
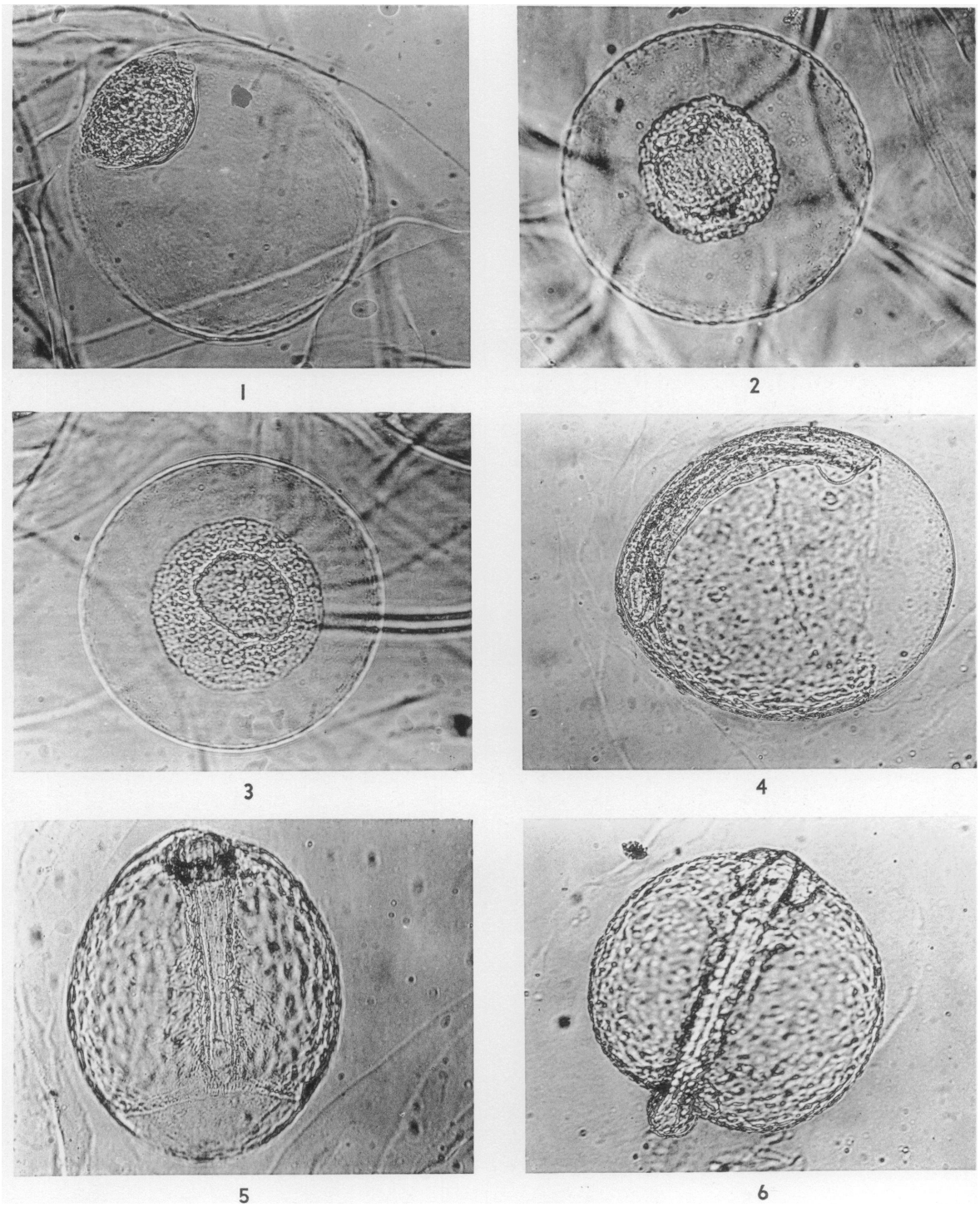
1. Stage 19, transverse section showing optic chiasma and developing rectus muscles
2. Stage 20, oblique section showing thin peritoneal membrane connected with cells of thick floor of pharynx
3. Stage 20, longitudinal section through pectoral fin, showing cartilage and evagination at extreme tip
4. Stage 20, transverse section showing layers of retina, infundibular region, and cartilage of parasphenoid bone
5. Stage 20, transverse section showing close association of liver and yolk, the gut, and the two pronephric ducts

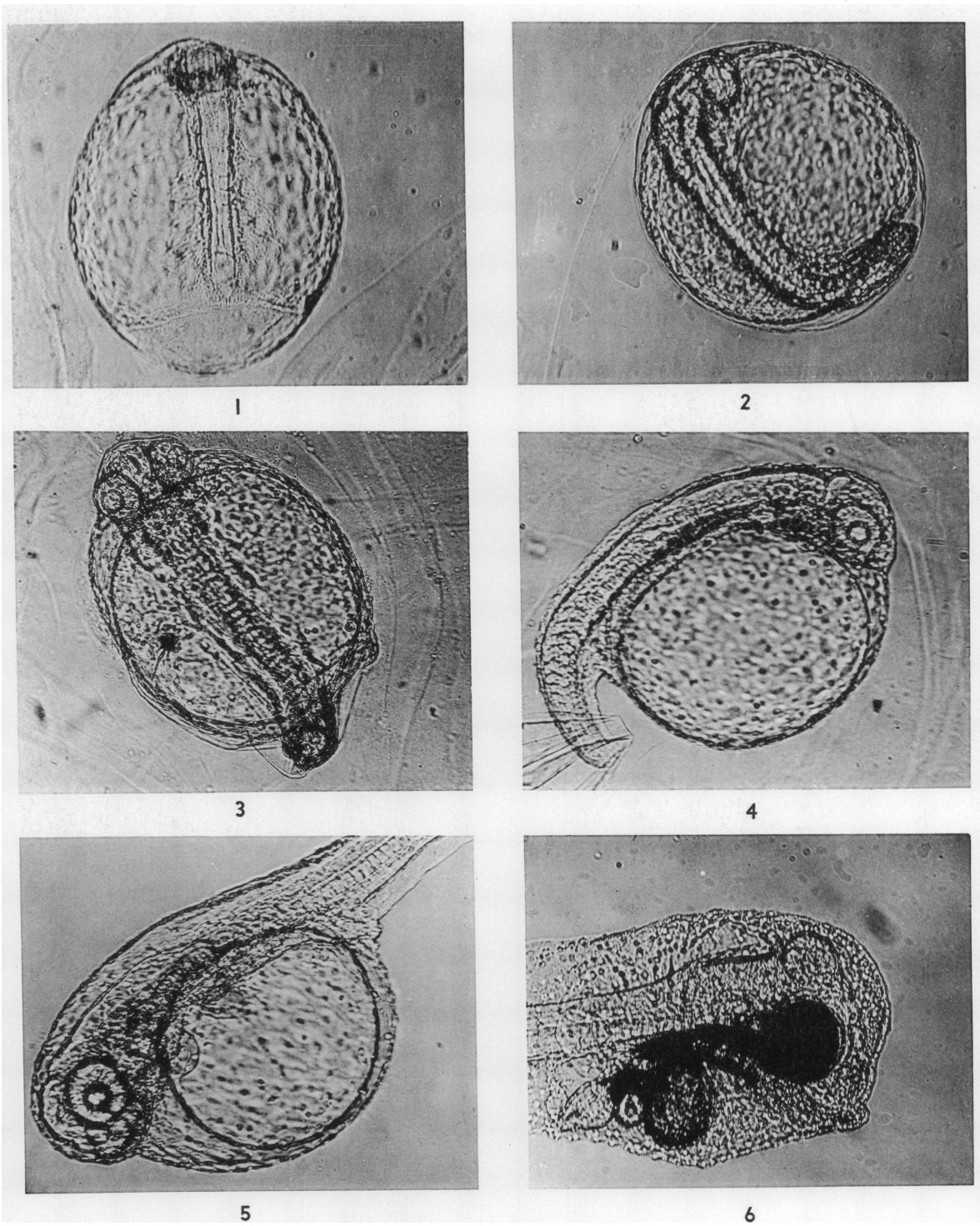


1. Stage 21, oblique section showing septum formed in otic capsule, cartilages of gill arches, and specialization of pericardium
2. Stage 21, sagittal section showing open mouth
3. Stage 23, transverse section showing hypothalamic region of brain and anlage of neurohypophysis above bifurcation of parasphenoid bone
4. Stage 23, transverse section showing incorporation of yolk and its capsule within the liver



DEVELOPING *Histrio* LARVAE

1. Late blastula, comparable to stage 9 of *Antennarius*
2. Stage 9, early germ ring
3. Stage 10, formation of embryonic shield
4. Stage 14, approximately 36 hours after spawning, showing optic vesicles, Kupfer's vesicle, and extent of periblast
5. Stage 15, showing appearance of somites
6. Stage 16, showing differentiation of lenses, developing gill slit, and auditory placodes



DEVELOPING *Histrio* LARVAE

1. Stage 16, showing somites and extent of periblast which does not yet completely encompass the yolk
2. Early stage 17, about two and one-half days after spawning
3. Late stage 17, embryo about to hatch, about three days after spawning
4. Late stage 17, hatching embryo
5. Stage 18 or 19, about four and one-half days after spawning, showing scarcity of pigment, believed to be caused by insufficient lighting
6. Stage 22, seven days after spawning, showing completion of retinal pigmentation and forward rotation of eyes