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THE EARLY EMBRYOLOGY OF TELEOSTS WITH SPECIAL REFERENCE TO PERCINA CAPRODES, BRACHYDANIO RERIO AND OSMERUS MORDAX

by

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CHAPTER I

INTRODUCTION

Present State of Knowledge

> Les Téléostéens sont parmi les Vertébrés ceux qui ont fait l'objet du plus grand nombre de recherches (Oellacher, Agassiz, Henneguy, List, M. von Kowalewsky, Whitman, plus recemment Kopsch, Boeke, Sumner, Lanzi, etc.).

With these words Brachet (1935) began his section on teleost embryology. Mansueti (1954) listed 882 references to papers dealing with this topic, and he stated that his survey covers only the teleosts found in Maryland. In view of this body of information on the embryology of fishes, particularly of teleosts, the following statement is astonishing:

> It [fish embryology] has not contributed very extensively to the development of the terminology and the basic concepts of vertebrate embryology (Witschi, 1956).

This statement implies that investigations in fish embryology have not produced results comparable in breadth to those on other vertebrates. Although the developmental peculiarities of hundreds of species have been studied, their broad significance has not been interpreted in the literature. As might be expected, this lack of interpretation is reflected in most contemporary textbooks of embryology, in which fishes are either ignored or treated as "interesting variants from

the prototype of primitive development" (Witschi, 1956). It also is reflected in the fact that embryology has contributed little to the interpretation of the phylogeny of fishes. These facts regarding the present state of knowledge of fish embryology lead to the conclusion that in spite of the large number of species studied, the known particulars have not been integrated into an organized body of knowledge.

Scope of Study

This paper represents an attempt to improve this situation. It deals mainly with teleosts, and is limited to their early embryology, the period from fertilization through gastrulation. This is the period in greatest need of integration. What Pierson (1953) expressed is still true:

> There seems to be very little agreement among investigators on related forms, especially from cleavage through gastrulation.

Development beyond gastrulation also needs clarification, but this period witnesses the formation of organ primordia of such complexity as to make its inclusion too extensive for this project. To follow any one organ and not all would detract from the emphasis on early embryology considered pertinent for this treatise.

Aims and Plan of Study

The three species chosen for this study are the northern logperch, Percina caprodes semifasciata (DeKay), the zebrafish or zebra danio

Brachydanio (or Danio) rerio (Hamilton = Buchanan) and the American smelt, Osmerus mordax (Mitchill). The principal aim was to discover basic features of early development in one species, the logperch, and to test their significance against comparable features in two widely different teleosts, the zebrafish and the smelt. An attempt has also been made to interpret early development of the Holostei as represented by Amia calva. Descriptive information on Amia was drawn from the work of Pierson (1953), which is the most extensive recently done on this species. The deficiencies in the literature on the early embryology of fishes are illustrated by a discussion of Pierson's (1953) report on gastrulation in Amia.

Terms are defined when it is necessary to emphasize fundamental concepts or when there is no consensus on meaning. At Dr. A. H. Stockard's suggestion, the term "gastropore" is substituted for "blastopore," as a more appropriate way of designating a structure associated with gastrulation. The terminology for the germ layers and their derivatives is that favored by Brachet (1935). Fertilization, segmentation, blastulation and gastrulation are called developmental phases and their subdivisions are referred to as stages. The term phase is chosen for these larger steps in order to reserve the term stage for the smaller steps, which usage is well established in the literature.

Chapter II deals with materials and methods. Its unusual length is necessary because of the number of species introduced and the special

problems of egg culture for each of the three species chosen for this study, and because of the inherent difficulties in the preparation of histological sections of fish embryos. Chapter III is devoted entirely to the problem of staging early embryos. This is a crucial problem in comparative studies; the lack of a standard system makes very laborious the interpretation of information found in the literature. A solution to this difficulty is proposed. Chapter IV covers the structure of the mature ovum and its development from fertilization through blastulation. Chapter V deals with gastrulation. Since this process cannot be described without considering morphogenetic movements, the work of Pasteels (1936) is used as a basis for following morphological progress in the species studied here. Finally, the nature of this work has led to some observations on dimorphism and on primitiveness in the logperch. These observations are reported in the Appendix.

CHAPTER II

MATERIALS AND METHODS

Choice of Species

The American smelt (Osmerus mordax), the zebrafish (Brachydanio rerio) and the northern logperch (Percina caprodes semifasciata)were chosen for two main reasons: they are in widely separate taxonomic positions, and they were easily available when this work was undertaken.

The American smelt. --Osmerus mordax, in family Osmeridae, represents the Clupeiformes (Isospondyli), a highly diversified order of soft-rayed teleosts that nevertheless are considered to be relatively primitive. This species also is readily available at Meach Lake, fifteen miles from the laboratory of The University of Ottawa. Figure 4 shows Meach Lake in the background, and in the foreground is the stream where the smelt eggs for this study were collected. Further details on the smelt and its breeding at Meach Lake are given by Legault (1956). In their review of the literature on the biology of the smelt, Marcotte and Tremblay (1948) state that very little has been published on its embryology.

The zebrafish. --This species, in family Cyprinidae, is a more specialized form of the soft-rayed teleosts. Being a domesticated species

that spawns throughout the year, it has obvious advantages over a wild species for embryological study. Native to India (Hamilton = Buchanan, 1822), the zebrafish was introduced as a pet into America more than fifty years ago. Its usefulness for teaching and research was suggested by Creaser (1934), Roosen-Runge (1938) and Rugh (1948). The extensive use of its eggs in experimental studies (Oppenheimer, 1936a; Battle and Hisaoka, 1952; Hisaoka and Hopper, 1957) and for biological assay (Jones and Huffman, 1957), is another reason for choosing it. After the beginning of this investigation Hisaoka and Battle (1958) published a paper on normal developmental stages of the zebrafish. This reference gives the reader an opportunity to evaluate the systems of staging that are commonly followed at present; hence an exhaustive survey of the literature on staging is omitted here.

The northern logperch. -- The selection of Percina caprodes semifasciata (family Percidae) as an example of the most highly evolved fishes, the spiny-rayed group, was inspired by Reighard (1913), who described the spawning of this subspecies on the shore of Douglas Lake, Cheyboygan County, Michigan. The presence of a spawning population of logperch (Figs. 2, 3) on this lake near the laboratories of the Biological Station of The University of Michigan also provided ideal circumstances for obtaining and studying its eggs during the summers of 1953-1956. The wisdom of choosing the logperch is supported by Winn's (1958) paper in which he concludes that this species is relatively primitive

among the fourteen species of darters he has studied. Observations and remarks concerning its primitiveness are presented in the Appendix.

Since this study is based mainly on the logperch, some salient points of its biology are reviewed. In spite of its color pattern, which closely resembles that of the yellow perch, Perca flavescens, the logperch does not belong to the same subfamily. It is a darter, subfamily Etheostomatinae, as is evidenced by the presence of such taxonomic features as the smooth free edges of the preopercle (Fig. 6), the weakly emarginated caudal fin and the low number of branchiostegal rays. Like other darters, the logperch uses its enlarged pectoral fins for several purposes. When at rest on the bottom of a protected niche, it uses them as support to keep the anterior portion of its body slightly raised, as shown in Figure 1. They also are mainly responsible for the characteristic quick jumps for which the members of this subfamily are collectively called darters. When frightened while resting on the bottom, individuals of this species on occasion have been seen to jump out of a foot-deep aquarium full of water. The habit of darting unexpectedly from a log-like posture (hence the name logperch) also is effective in capturing swift prey. A motion picture film taken of captive specimens leaves no doubt of the efficient use of the pectoral fins in capturing minnows. These fins also are used to unearth food by fanning the bottom sediment away, and individuals have been observed to bury themselves by fanning up of the sediment or sand, until only the eyes were exposed, a habit

possibly useful in both protection and aggression.

Bailey and Gosline (1955) gave the genus Percina a prominent place by bringing into that genus about 20 species of darters with between thirty-eight and forty-five vertebrae. These darters also have in common such other features as a terminal or subterminal mouth, a complete lateral line, and pelvic fins not inserted close together. The mid-ventral file of many-pointed and enlarged scales supposedly typical of this genus is discussed in the Appendix.

In the Great Lakes region, where the specimens for this study were collected, the genus Percina is represented by seven usually recognized taxa. P. caprodes is distinguished from the others by a subterminal mouth, sixteen narrow, vertical, dusky bars, and usually more than 85 scales in the lateral line.

The prevailing common name of this species is logperch (American Fisheries Society, 1960), but it is also known locally as hog-fish, stone-roller, zebra darter, Modoch, Manitou darter and pike-minnow. Two Great Lakes subspecies are recognized, which Hubbs and Lagler (1958) characterized as follows:

> Nape closely scaled, bars more even and regular, scarcely expanded into blotches in a median lateral row. OHIO LOGPERCH--Percina caprodes caprodes (Rafinesque).
>
> Nape with a triangular scaleless area; bars less even, and often expanded into a medial lateral row. NORTHERN LOGPERCH--Percina caprodes semifasciata (De Kay).

The northern logperch is the only form found at Douglas Lake. The Ohio logperch is found more southward, and intergrades are found throughout much of northern Ohio (Trautman, 1957).

Culture Techniques

Since the techniques for obtaining the embryological material had to be adapted to the natural history of each of these forms, the methods devised for each are described.

For the logperch. --The eggs of the logperch are not easily collected on the spawning grounds, and the few that can be so obtained are not in favorable condition for embryological study. They are demersal and adhesive and soon become hidden in the substratum by the sand grains and detritus that adhere to them. The layer of extraneous material on the eggs usually is so dense that the few eggs painstakingly collected do not reveal any of their internal structure when observed under the microscope. Decapsulation would overcome this inconvenience but is not practicable because the eggs burst when decapsulated before gastrulation is complete.

To obviate these difficulties, breeding adults were collected and the eggs were artificially fertilized. Breeding schools were trapped by encircling them with a 20' x 4' x 1/4"-mesh Common Sense minnow seine. The specimens were sorted according to sex and the males and females were kept in separate concrete laundry tubs supplied with running well-or lake-water.

Because of the small amount of milt obtainable from each male, a modification of the Russian or "dry" method was devised for fertilizing the eggs. The testes of one male were macerated in a moistened Syracuse dish and about 60 eggs were then stripped into it. The eggs were permitted to remain in the macerate for 15 minutes, after which they were washed with fresh water into a finger bowl in such a way that they would not strike each other but would spread evenly on the bottom of the bowl. Non-adhesive while in the macerate, the eggs immediately become adhesive and cling together or to the dish when the macerate is washed out with fresh water. They were then thoroughly rinsed and 100 cc. of fresh lake-water was poured into the finger bowl.

Out of the 273 strippings on which attempts to obtain fertilization through such procedure were made, 198 were from 10% to 90% successful, but 75 attempts were wholly unsuccessful. Complete failure in 27% of the attempts might be attributed to the possibility that the eggs were either immature or overripe. Attempts to clarify this point were made on the eggs of 30 females, stripped five days after capture. No difference was found in the color, shape and transparency of the eggs, and yet fertilization completely failed in 16 and occurred in only 14 of the attempts. However at that time (1955), no observation was made on the color of the ovipositor, which later was found to be clear in ripe female darters (Strawn and Hubbs, 1956). Since the state of ripeness of the eggs could not be determined, it is difficult to evaluate the fertilization technique described above.

Among the 198 cases in which any fertilization resulted, the number of fertilized eggs varied from 10% to 90%, as stated above, with an average of a little over 50%. Using the "dry" method, Strawn and Hubbs (1956) reported a range of 60% to 80% for darters in general, but they did not give specific figures for the logperch.

The incubation technique was similar to that later described by Strawn and Hubbs (1956). Infestation by Saprolegnia was quite common and could not be checked by the prophylactic and curative methods recommended by Rugh (1948). Infected eggs were pipetted from the culture, or the culture was discarded. This problem led me to experiment with trout eggs. Fifty thousand rainbow trout eggs were treated with Malachite green, as advocated by Foster and Woodbury (1936). This treatment, accompanied by controls, was found to prevent the appearance of the fungus.

The collection of preserved material for the logperch contains 10 specimens for each of the 70 stages arbitrarily chosen to represent development up to 10 days after hatching. Thirty-four of these stages cover the period extending from pre-fertilization to the closure of the gastropore.

For the zebrafish. --The methods used for handling zebrafish material were substantially the same as those used for the logperch material, except for the securing of fertilized eggs. The technique for

securing zebrafish eggs was a refinement of those described by Creaser (1934) and Roosen-Runge (1938).

The control of the breeding activities of the zebrafish is based upon the fact that under otherwise similar conditions, these fish spawn later in the day in winter than in summer (Roosen-Runge, 1938). The delay in winter is related to the delay in arrival of daylight. Taking advantage of this photoperiodicity, and artificially prolonging the dark period by keeping the aquarium covered until the eggs were wanted (Legault, 1958, 1960), schools of 48 to 60 fish kept in 24" x 12" x 12" aquaria in a ratio of two males to one female were induced to yield crops of 50 to 100 eggs each morning at any set time.

Zebrafish, like logperch, will devour their eggs. However, because zebrafish eggs are demersal but not adhesive, they are more easily protected by artificial means. From the amateur fish breeders I learned a successful technique of covering the bottom of the aquarium with 1/2" marbles and removing the eggs from between them with a pipette. This technique was refined for mass production in the laboratory.

A siphon was substituted for the pipette and a screening apparatus was added to the system (Figs. 10, 11). The apparatus consisted of a detritus trap made of screen wire of mesh large enough for the eggs to pass through, and of an egg trap consisting of a wire tea strainer of mesh small enough to hold the eggs. After the eggs had been collected on the tea strainer, they were carefully washed into a finger bowl. Water

was then poured into the aquarium to restore the original level. To reduce the amount of detritus siphoned with the eggs, the marbles were covered between the spawning periods with cheesecloth sewn onto a wire frame.

To limit the area over which eggs were deposited and reduce the siphoning to a minimum, a two-level bottom was constructed in the aquarium (Fig. 7). A window-glass partition 5" high was set vertically lengthwise of the center on the bottom of the aquarium, and securely fastened in position. The area in front of the partition was covered with a single layer of multicolor marbles, and the rear half of the aquarium was filled to the top of the partition with white sand. Spawning regularly occurred in the deeper water over the marbles but was never observed in the shallow water over white sand. However, spawning took place on the sand shelf when white sand was replaced by a multicolor one.

The specimens collected represent 30 stages, 20 from early embryology and 10 from later development.

For the smelt. --Naturally-fertilized smelt eggs were easy to collect. They were deposited in a stream less than a foot deep where they adhere to pebbles or detritus that could be transferred to aquaria with the eggs attached. However, certain problems arose in the culture and study of these naturally-fertilized eggs, and experiments were undertaken to avoid or control such undesirable factors as crowding of eggs on the pebbles or detritus, and subsequent interference with microscopic

study by detritus. A suitable quality of running water in the laboratory also was a problem to be solved. These problems were effectively solved or averted in the following manner:

In the spring of 1960, ripe males and females were collected with a dip net and put into moistened 24" x 12" plastic bags, in which they discharged eggs and milt almost immediately. The adult fish were then removed and half an hour later the mass of eggs and milt in the plastic bags was diluted with an equal volume of stream water. After remaining in this mixture an hour, the eggs lost their adhesive properties and, therefore, were much easier to handle. The milt was then washed out and the eggs were transferred to 6" x 12" plastic bags. Approximately 200 eggs and enough stream water to partially submerge them were placed into each bag. The bags were then inflated with oxygen and incubated at 45° F. Water and oxygen were renewed at 48 hour intervals. The eggs developed normally with less than 10% mortality, and no fungus appeared. Stages ranging from blastula to closure of the gastropore were collected, and the remainder was discarded after the embryos had reached the ten-somite stage. This method was used only once and not enough evidence was obtained to assess its full merit.

Other Techniques

Histological technique. --Although the eggs of the logperch, zebrafish and smelt all presented different problems of culture, they all showed

unusual difficulties in the preparation of serial sections. Indeed, they and those of all other teleosts are heavily yolked, belonging to the category of eggs which Gray (1954) characterized as follows:

> Heavily yolked embryos are among the most difficult objects from which to prepare satisfactory serial sections, as may be witnessed by the photographs which illustrate the work of many experimental embryologists.

The difficulty stems from excessive hardening of the yolk. This occurs in eggs treated with such routine fixatives as Stockard's solution, Bouin's, Bouin's-dioxane, Bouin's plus 1% urea, and Lavdowski's mixture. On the other hand, Gregg and Puckett's and Smith's fixatives, so highly recommended by Gray (1954) for such material, require a rather lengthy procedure. This is a handicap in preparing early stages of such rapidly developing eggs as those of the logperch and zebrafish. The short time during which logperch eggs can be collected and the necessity of decapsulating them for rapid fixation and accurate orientation during embedding also make these fixatives difficult to use.

The faster fixing procedure described below was found to give satisfactory results when sections of the yolk are not required. The eggs were put into hot (45°-50°C.) Bouin's for four minutes, for the purpose of hardening them enough to make decapsulation possible. After decapsulation, the eggs were returned to the fixative for 10 minutes, which is sufficient to fix the embryo entirely and the yolk partially. After bleaching in 70% ethyl alcohol saturated with lithium carbonate, the specimens were sorted by stages into cheesecloth bags and stored in

1-pint Mason jars of 80% ethyl alcohol.

To facilitate orientation while embedding the eggs, they were stained in toto with eosin or Grenacher's alcoholic borax carmine and differentiated with acid alcohol until the embryo alone retained the stain. The eggs were embedded in liquid Tissuemat (56°-58°C.) after 90 minutes of treatment, and sectioned at 10 microns with a mechanized microtome (Fig. 15). To avoid loss of sections, the mounted slides were dipped in a 5% solution of celloidin in a 1:1 mixture of ethyl alcohol and ether. Among the seven different stains tried for differentiating cell borders in early stages, picro-spirit blue (Smith, 1912) was found to be the most satisfactory, especially with zebrafish embryos. The generally poor staining results that were obtained might be attributable to the fixative used.

The serial sections thus prepared include cross and longitudinal sections of 60 stages for the logperch, a set of 20 zebrafish early embryos, and stages for the smelt ranging from blastula to closure of the gastropore. Comparison of Figures 60, 64 and 65 shows that sectioning of the yolk in the logperch presents special problems that were not completely solved by the technique described above.

Photographic technique. --The following topics for the logperch were recorded on sixteen millimeter color film with a Cine-Kodak camera: spawning behavior, seining of a breeding school, artificial fertilization, and predation on live minnows. Photomicrographs of

almost all early stages of living logperch were taken with a Leica camera. The main characteristics in many instances could not be recorded on photographs for such reasons as light interference from the chorion, free rotation of the egg within the chorion, small size and transparency of the structure sought, and technical difficulties in decapsulating the living egg. Attempts to use preserved material were not successful because no technique was found to solve all the problems mentioned above. The photomicrographs served as a basis for the preparation of Figures 16 to 33. Lewis and Roosen-Runge's (1944) film on early development of the zebrafish was found to be extremely useful for studying and interpreting the oosplasmic and cellular movements observed in living eggs.

Drawing technique. --All original drawings for Chapters III to V are diagrammatic: they are intended to help the reader visualize the essentials rather than the details of morphogenesis of the logperch at a particular stage. The series on developmental stages (Figs. 16-33) is based on photomicrographs of living embryos and on observations from whole mounts and sections. The series on the formation of the periblast (Figs. 34-49) and that on gastrulation (Figs. 75-84) are based on reconstructions made from paper cut-outs and wax pencil drawings on frosted glass plates. This procedure was employed because of the difficulty in obtaining an ideal plane of section with such small eggs, and because of the artifacts commonly encountered in sections of fish eggs.

CHAPTER III

SYSTEM OF STAGING EARLY EMBRYOS

Analysis of Existing Systems

One of the major problems in the literature on early embryology of fishes is the unreliability of existing systems for identifying comparable stages in their development. Evidence for this statement is drawn from an analysis of the four systems used by Hisaoka and Battle (1958) for staging zebrafish embryos. These systems are:

The numerical system. --For the period extending from fertilization through gastrulation in the zebrafish, Hisaoka and Battle (1958) present sixteen stages, numbered one to sixteen. Their Table 1 shows that they, Oppenheimer (1937) and Tavolga and Rugh (1947) did not use identical serial numbers in their work on Brachydanio rerio, Fundulus heteroclitus and Platypoecilus maculatus, respectively. For instance, Hisaoka and Battle's stage 12 in Brachydanio becomes Oppenheimer's stage 11 in Fundulus and Tavolga and Rugh's stage 4 in Platypoecilus. The same table also shows that one author may use different numbers for similar stages in different species. For instance, Hisaoka and Battle's (1958) stage 12 is Battle's (1940) stage 5. These facts indicate that in the numerical system of staging embryos, the stage numbers have

been used not with standard meaning but rather to indicate the relative position of a particular stage in the total number of stages studied by that author in that species. The basis of the system, therefore, is subjective and it has no value for comparative studies.

The chronological system.--The chronological system of staging embryos consists of identifying them by their age in hours. Table 1 shows that the age in hours at hatching time varies from species to species.

TABLE 1

AGE IN HOURS AT HATCHING FOR SOME SPECIES OF TELEOSTS

Species	Incubation Temperature in C^{o}	Age in Hours at Hatching	Source of Information
Brachydanio rerio	26^{o}	96	Hisoaka and Battle (1958)
Fundulus heteroclitus	25^{o}	265	Oppenheimer (1937)
Carassius auratus	18.5^{o}-29.5^{o}	72-96	Battle (1940)
Xiphophorus maculatus	. . .	120-192	Rugh (1948)

Table 2 shows that the age at hatching varies in Brachydanio rerio. However, the gap between the findings of Hisaoka and Battle (1958) and of Battle and Hisaoka (1952) seems excessive. Rugh (1948) states that

in Xiphophorus maculatus, "the variation proved to be so great that an estimation of the true chronological age was impossible." These facts indicate that the rate of development is highly variable in teleosts. Consequently, the chronological system of staging is unreliable in comparative studies.

TABLE 2

AGE OF BRACHYDANIO RERIO AT HATCHING

Incubation Temperature in C°	Age in Hours at Hatching	Source of Information
27°	76	Creaser (1934)
26.5°	60	Roosen-Runge (1938)
26°	96	Hisaoka and Battle (1958)
25°	144-240	Battle and Hisaoka (1952)
25°-27°	72-108	Original

The nominal system. --Table 3 shows that in the nominal system the names given to stages are descriptive: they tend to place stages among the four major phases of early development. The names given to stages 3 to 8 clearly indicate that they belong to the segmentation phase. The terminology used in the sequence of stages 1 to 8, that is egg, blastodisc, ovum, and late cleavage stages, is not consistent. Moreover, the name, late cleavage stages, is vague in comparison to the names used for the preceding stages. According to Hisaoka and

TABLE 3

IDENTIFICATION NUMBERS AND NAMES FOR SIXTEEN STAGES IN BRACHYDANIO RERIO (AFTER HISAOKA AND BATTLE, 1958)

Stage Number	Stage Name
1	Recently fertilized egg
2	1-celled blastodisc
3	2-celled ovum
4	4-celled ovum
5	8-celled ovum
6	16-celled ovum
7	32-celled ovum
8	Late cleavage stages
9	Early high blastula
10	Late high blastula
11	Flat blastula
12	Very late blastula
13	Early gastrula
14	Blastoderm enveloping 1/3 yolk sphere
15	Blastoderm enveloping 1/2 yolk sphere
16	Blastoderm enveloping 3/4 yolk sphere

Battle's description, the late cleavage stages could be called "64- to 256-celled ova." The names for stages 9 to 12 place them in the blastulation phase. The adjectives used for qualifying the blastula are vague for both morphological and chronological use. The descriptive value of these adjectives is very limited, therefore, in comparison to that of the adjectives used for qualifying the ovum at stages 2 to 7. From its name, stage 13 is definitely in the gastrulation phase, but the captions for the later stages do not so clearly indicate the phase to which they belong, because names for them are replaced by phrases describing an unreliable criterion for indicating the degree of progress of gastrulation in different species of teleosts. The reasons for its lack of reliability among teleosts will be discussed in the chapter on gastrulation. In brief, however, there are variations in the extent to which the blastoderm has enveloped the yolk at the end of gastrulation. Nevertheless this feature may be useful for identifying stages in a particular species, especially if its relation to the termination of gastrulation is indicated. Battle and Hisaoka (1952) follow this procedure when they state that the closure of the gastropore indicates the termination of gastrulation in the zebrafish. However, Hisaoka and Battle (1958) do not specifically correlate the two in their description, but in their summary they dispose of their stage 17 (closure of the gastropore) by stating that "gastrulation is represented by 4 stages (13-16)." By using different landmarks in their two papers they raise two questions: what is meant by termination

of gastrulation? how is it recognized?

This analysis of the nominal system shows it to be inadequate in its present form. It often is inconsistent, vague and misleading and does not always reflect the true morphological progress of the embryo toward the adult-form. Because it is not based entirely on the fundamentals of embryology, its value in comparative studies is limited.

The short stage - description system. --Short descriptions of stages usually are added to compensate for the deficiencies in the existing systems of staging embryos. The limitation in the value of these descriptions in comparative studies is indicated in the remark made by Hisaoka and Battle (1958) regarding the comparison of stages they established for the zebrafish with those established for related species. They remark that the comparisons are valid "insofar as comparisons are possible from the descriptions and figures of the three species presented by the different investigators." This implies that the meaning of these short descriptions varies from one investigator to another. Indeed Table 4 shows that the conclusions I drew from some of the descriptions made by Oppenheimer (1937) and Tavolga and Rugh (1947) do not agree with the conclusions that Hisaoka and Battle drew from the same descriptions.

It is difficult to establish the norms governing the amount and quality of information to be included in short descriptions of most developmental stages. Indeed, morphogenesis is a cable of many

parallel chains of complex changes whose links join and overlap into one another without any real point of complete demarcation. Major trends are obvious: over any given period of time ontogenetic processes tend to achieve some major intermediate goal toward the adult form. The series of these intermediate goals forms a natural basis for separating morphogenesis into phases. Such events as fertilization, segmentation, blastulation and gastrulation are natural phases, but division of these phases into stages is more difficult. When this is attempted, it soon is realized that the intricacy of ontogenetic processes obscures all natural landmarks making it necessary to resort to artificial dissociation. Choice of artificial landmarks may vary according to the viewpoint of the investigator, who may be an ichthyologist, a comparative embryologist, an experimental embryologist or a student of natural history. Moreover, any investigator under such circumstances may rightly be a "splitter" or a "lumper" in dissociating processes, provided he defines his terminology.

Under the circumstances indicated above, the norms governing the type of information essential to short descriptions of stages cannot be established until understanding of and agreement on the artificial aspects of our method of analyzing development have been reached. Actually, the lack of consensus is the core of the difficulties encountered in interpreting the literature on early embryology of teleosts.

Proposed Decimal System

Its basis. --The essential basis for a valid system of staging is the agreement among investigators on the natural and artificial criteria by which developmental progress may be dissociated into parts. Such agreement for staging the early teleost embryos has not been reached, but the system proposed here is based on what seem to be acceptable criteria. It provides for the division of the early embryology of teleosts into four phases named and numbered from one to four, as follows: 1) fertilization, 2) segmentation, 3) blastulation and 4) gastrulation. Phase 1, fertilization, includes activation of the egg, syngamy of the two pronuclei and organization of the zygote. The two most striking morphological achievements during this phase are the lifting of the egg membrane and the formation of the blastodisc. The extent of their progress is used to characterize the stages within the phase. Under normal conditions this phase begins the instant a sperm cell makes physiological contact with the surface of the egg and ends as the first cleavage begins.

Phase 2, segmentation, is the phase during which the most striking progress toward adult form is the increase in the number of cells without substantial increase in the volume of the blastodisc. Since the increase in cell number is achieved by consecutive and more or less synchronous mitoses, the number increases approximately by geometrical progression with a mode of two. Accordingly, the main stages of segmentation are those in which the numbers of cells in the blastodisc

successively agree with the figures in this progression. Segmentation begins with the first mitosis and terminates with completion of the ninth cleavage. Segmentation continues after this level of organization is reached, but its characteristics soon are overshadowed by those of blastulation, the next phase of development. The practical reason for advocating the ninth cleavage as the end of the segmentation phase is the fact that in the teleosts I have studied this is the last stage in which the number of cells can be estimated in life under x50 magnification.

Phase 3, blastulation, begins with the tenth cleavage and ends with the appearance of first evidence of involution at the dorsal lip of the gastropore. During this phase the processes of segmentation are overshadowed by changes whereby the originally hemispherical blastodisc takes the shape of an eccentric concavo-convex lens. In the course of this remodelling, the blastodisc assumes shapes that easily can be referred to common geometrical figures, shapes that are proposed as arbitrary landmarks for stages of blastulation.

Phase 4, gastrulation, is the phase during which an embryo transforms from a blastodisc with an eccentric axis into one with a longitudinal axis. This is accomplished by specialized cell movements that also are responsible for the establishment of the germ layers. It is proper, therefore, to take the first evidence of gastrular movements as a landmark for the beginning of the gastrulation phase. The last stage of this phase is marked by the appearance of a keel, but there is still no evidence of neurulation.

TABLE 4

COMPARISON OF STAGE NUMBERS USED BY VARIOUS AUTHORS

Stage No.	Stage Characteristic	Brachydanio rerio (Hisaoka and Battle, 1958)	Fundulus heteroclitus (Oppenheimer, 1937)	Platypoecilus maculatus (Tavolga and Rugh, 1947)	Amia calva (Pierson, 1953)
0.9		1	1	1	-
1.6	Blastodisc formation begins	-	-	-	-
1.9	Blastodisc formation ends	2	2	-	-
2.0	First cleavage furrow	-	-	-	-
2.1	2-celled blastodisc	3	3	-	2
2.2	4-celled blastodisc	4	4	-	3
2.3	8-celled blastodisc	5	5	-	4
2.4	16-celled blastodisc	6	6	-	5
2.5	32-celled blastodisc	7	7	-	6
2.6	64-celled blastodisc	-	8	-	7
2.7	128-celled blastodisc	-	-	-	8
2.8	256-celled blastodisc	-	-	-	-
2.9	512-celled blastodisc	9	-	-	-
3.0	Hemispherical blastodisc	10	9	-	-
3.2	Biconvex blastodisc	-	-	-	-
3.4	Plano-convex blastodisc	11	10	-	-
3.6	Concavo-convex blastodisc	12	-	-	-
3.8	Eccentric blastodisc	13	11	-	12
4.0	Beginning of involution	14	12	4	-
4.5	Embryonic shield	15	-	5	13
4.7	Indented embryonic shield	-	-	-	14
4.9	Embryonic keel	16	-	6	15

A summary of the characteristics proposed above for staging early embryos of teleosts is presented in Table 4.

Mechanics of the system. --The proposed staging system for teleosts consists of expressing both the phases and the stages suggested in the preceding section with numerals. The sequence of phases is expressed as follows: Numeral 1 stands for the fertilization phase, 2 indicates segmentation, 3 designates blastulation and 4 is used for gastrulation. Zero, therefore, would designate the pre-fertilization phase. The subdivision of phases into stages is expressed by assigning each stage the number of the phase in which it falls and qualifying it with a decimal digit or digits appropriate to the stage. Later stages within a given phase are assigned correspondingly higher decimal numerals. For instance, 2.1 is the stage of the segmentation phase in which the first cleavage has been completed and 2.5 is the stage of segmentation in which the fifth cleavage has been completed. Table 4 presents the numbers and the characteristics for 22 stages of the early development of teleosts. The following application of this system to the logperch more fully illustrates its use.

Its application to the logperch. --The purposes of this section are to supplement the information given on the main characteristics of each stage, to describe the mode of these characteristics, and to give techniques for recognizing them in life under x50 magnification. The main characteristics are further emphasized in Figures 16 to 33.

Stage 0.9 is the last stage of the pre-fertilization (zero) phase, when the ovum is ready for fertilization. The three main components of the ovum at this stage are concentric spheres, closely adhering to each other, the yolk in the center, the egg capsule on the exterior, and the ooplasm between them. The egg of the logperch is about 1 mm in diameter, its yolk is colorless, and superficially embedded in the yolk is a pale yellow oil globule .41 mm. in diameter. Observation of living eggs at x50 magnification did not yield any reliable criterion for estimating their degree of maturation.

Stages 1.0 to 1.9 cover the phase of fertilization. As previously mentioned, the stages within this phase are characterized by the extent to which the egg membrane is lifted and by the form of the blastodisc. In the logperch it was observed that shortly after milt comes into contact with the egg, lacunae appear between the ooplasm and the egg membrane. This feature is chosen to characterize stage 1.1, and number 1.7 is assigned to the stage when the membrane is lifted to its maximum height. The blastodisc begins to form before stage 1.7; its beginning is chosen to characterize stage 1.6, and number 1.9 designates the stage when the blastodisc is completely formed. Figure 16 shows the relative positions of the ooplasm and yolk at stage 0.9, and Figure 17 shows them at stage 1.9.

Stages 2.0 to 2.9 cover the segmentation phase. Stage 2.0 should be characterized by the prophase of the first cleavage but, because phases

in mitoses are not easily detected in life at x50 magnification, this stage is determined by the appearance of the first cleavage furrow. The resting stages after the completion of the first nine cleavages are numbered 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, respectively. In the first four of these stages the cells are hemispherical and loosely held together, with their floors resting on the spherical yolk mass. Cell counts are best made from polar views, but this requires some handling of the egg because in life the blastodisc faces laterad and the embryo rotates freely within the capsule.

The numbers of cells expected for stage 2.5, 2.6, 2.7, 2.8 and 2.9 are indicated in Table 5 but, because of asynchronous cleavage, these ideal numbers were not encountered in the logperch. These stages therefore are more loosely characterized to include blastodiscs whose cell numbers approximate the ideal. Accurate cell counts are difficult if not impossible to make on living material. Table 5 gives useful information for estimating the total number of cells in the logperch blastodisc. The arc cells referred to in this Table are shown in Figures 22 to 25 as the peripheral cells along an arc passing through the pole and opposite points on the margin of the blastodisc. The figures for the total number of cells in the blastodisc were obtained from counts made on serial sections.

TABLE 5

RELATION BETWEEN THE NUMBER OF ARC CELLS AND THE TOTAL NUMBER OF CELLS IN THE BLASTODISC OF P. CAPRODES (STAGES 2.5 TO 2.9)

Stage	Number of Arc Cells	Total Number of Cells in the Blastodisc	Ideal Number of Cells
2.5	7	35	32
2.6	13	60	64
2.7	16	122	128
2.8	21	268	256
2.9	25	528	512

Stages 3.0 to 3.9 cover the blastulation phase. Stage 3.0 is a hemispherical blastodisc with a cell count higher than 800. The blastodisc changes successively from the hemispherical form to biconvex (stage 3.2), to planoconvex (stage 3.4), to converging concavo-convex (stage 3.6), and finally to an eccentric form of the last (stage 3.8). These stages are illustrated in Figures 26 to 30. Blastulation and segmentation stages superficially resemble each other but can be distinguished by cell size. Stage 3.0 can be distinguished from stage 3.4 by comparing the length of the geometrical radii of the blastodisc. The stage 3.0 blastodisc is hemispherical and all its radii are approximately the same length, but in the stage 3.4 blastodisc, which is relatively flat, the equatorial radii are recognizably longer than the polar ones. Throughout blastulation the true shape of the floor of the blastodisc

is easiest seen through the vegetal pole of the egg.

Stages 4.0 - 4.9 cover the gastrulation phase. Stage 4.0 differs from stage 3.8 by having an opaque spot in the transparent rim of the blastodisc. This spot marks the beginning of involution, at the dorsal lip of the gastropore. The subsequent gastrulation stages are identified by the progress toward longitudinal axiation. At stage 4.5 the embryo is a conspicuous opaque shield within the otherwise translucent blastodisc. The shield is bilaterally indented at the tip in stage 4.7 and at stage 4.9 the shield has become a "keel." Closure of the gastropore is not considered to mark the end of gastrulation for reasons given in the chapter on gastrulation.

Discussion

Validity of the stage characteristics. --A theoretical foundation has been laid for the choice of the stage characteristics listed in Table 4, and an easy method for detecting them in the logperch also has been reported. Their reliability for use among teleosts as a group is enhanced by their presence in the zebrafish and the smelt, and also in the species studied by other investigators and listed in Table 4. In this table the stage numbers indicate that the investigators use the characteristics listed on the left of the table, and the dashes indicate that the investigators either omit the stage or characterize it differently. None of these authors gives any characteristic whereby the ripeness of the egg can be

determined under gross observations. Since I have not found one in the logperch either, I have not characterized stage 0.9 in Table 4. Stages 1.6 and 2.0 are not described by the authors mentioned in Table 4. Hisaoka and Battle's description for stage 8 in Brachydanio rerio contains the characteristics of my stages 2.6, 2.7 and 2.8. Oppenheimer's (1937) stage 8 for Fundulus heteroclitus includes the characteristics of my stages 2.6 to 2.9, but the characteristics for my stages 3.2 and 4.7 were not found in the descriptions of the authors quoted in Table 4. I easily detected these features in Brachydanio rerio, and for Fundulus and Platypoecilus, there is at least the fact that the descriptions do not contain anything contrary to the character descriptions I have employed. Tavolga and Rugh's (1947) work is included in Table 4 for the support it gives to the choice of characteristics for gastrulation stages: their stages 2 and 3 are vaguely characterized as segmentation and blastulation stages. Since most of the characteristics listed in Table 4 also are recognized by other investigators, they are proposed as useful criteria in the identification of stages during the early development of all teleosts.

The work of Pierson (1953) on Amia calva does not give all the information necessary for firmly establishing the presence of geometrical pattern similar to those proposed for staging blastulation in teleosts, but it does give some evidence that these criteria could apply also to holosteans. Even though the characteristics listed in Table 4 are not

envisioned to be valid for groups other than teleosts and holosteans, they could serve as a basis for establishing more definite and uniform standards in other groups of vertebrates.

Choice of stage numbers. --As previously mentioned, the decimal digits in this staging system increase in value for later stages of a phase. The present series of numerals contains gaps. The gaps indicate intervals between the stages that were characterized, and can be filled when more detailed study is completed. For instance, the ten fertilization stages described by Roosen-Runge (1938) for the zebrafish could be numbered 1.0 to 1.9. If finer staging were needed, second order decimals, such as 1.55 between 1.5 and 1.6, and also higher orders could be employed. The numerals proposed for the segmentation stages form a complete series, but those for blastulation were chosen to divide the unit into only five equal parts, the number of clearly characteristic stages, and the numerals for gastrulation were chosen to divide that unit into three main parts and an additional intermediate part between stage 4.5 and 4.9.

Whatever the total merit of this numeral system, the important point is that the numbers have standard values. Numerals of standard meaning would enable investigators to report more precisely on normal developmental stages of individual species and would eliminate the need for publishing comparative tables such as Table 4; it then would be sufficient to provide the reader with a list of the numbers of the stages studied, plus descriptions and numbers of any new stages proposed.

CHAPTER IV

FROM FERTILIZATION TO BLASTULATION

The aim in this and the next chapter is to reconstruct the continuous progress of the embryo toward adult form. Development is essentially action and cannot be described in terms of fixed stages. The developmental histories of the logperch, zebrafish and smelt are here considered in the light of the known facts for all teleosts, and the gaps in my observations are bridged with facts reported by previous workers. Comparisons with salient features in Amia, as described by Pierson (1953), are added whenever they are pertinent.

The Ovum

In order to find a basis for correlating and grouping the isolated facts obtainable from observations on a mature ovum (stage 0. 9), let us briefly recall the fundamentals of its architecture. Prior to fertilization the mature ovum already has undergone some drastic transformations: it no longer is a rudimentary sex cell (stage 0. 0) but rather is a differentiated mass of protoplasm capable, after proper fertilization, of multiplying and eventually differentiating into an adult form in the lineage of its ancestors. The growth and maturation processes have provided the unfertilized ovum with enough nutrients (yolk) to support

development to hatching, and also to produce membranes for protection of the embryo during the same period. The mature ovum therefore is made up not only of its protoplasm but also of two other main components, deutoplasm and a protective capsule. Each of these components was studied in the logperch, the zebrafish and the smelt.

The logperch. --The egg of this species (Fig. 34) is a nearly spherical mass about 1 mm. in diameter and is demersal. Except for a bulge around the nucleus, the translucent non-pigmented ooplasm forms a uniform thin layer around the deutoplasm. The latter is a spherical mass composed of light gray microscopic granules around a single, pale yellow oil globule about .41 mm. in diameter.

The egg capsule (Fig. 13) closely adheres to the outer surface of the ooplasm. It consists of an inner non-cellular layer of radiate appearance, presumably derived by secretion from the ooplasm, and an outer cellular layer, presumably derived from the ovary. As shown in Figure 61, these two layers undergo changes early in development. The inner layer loses its vertical and horizontal striations, and the outer one loses its cellular structure and becomes flat. Both layers become somewhat rigid and pressure resistant. Near the nucleus there is in the capsule a funnel-shaped microscopic opening, the micropyle, for admission of sperm cells. The outer surface of the capsule is adhesive when the egg is laid, but this property usually is lost in areas which do not make immediate contact with extraneous solid matter. However, in

10 cultures out of 103 observed for this factor, the entire capsule retained its adhesiveness until hatching time, but the larvae in all cultures looked perfectly normal. The significance of this difference in the loss of adhesiveness is not known.

The zebrafish. --The egg of the zebrafish is .625 mm. in diameter. Its three main components differ from those of the logperch in the following respects: the deutoplasm contains lacunae with some ooplasm, and as is shown in Figure 14, it consists of particles of various sizes and shapes which are not arranged in any apparent order. There is no large oil droplet, and there seem to be no small ones. The outer surface of the capsule is not adhesive when the egg is first deposited and remains so afterwards.

The smelt. --The smelt egg is about .85 mm. in diameter. Its deutoplasm differs from that of the logperch in that the yolk particles are intermingled with over a hundred small oil droplets. Comparison of Figures 12 and 14 shows that the particles of deutoplasm in smelt eggs are quite similar in shape, size and distribution to those in zebrafish eggs, except those in smelt eggs appear to be granular.

Even though the capsule externally is not adhesive at stage 0.9, it eventually does become a holdfast organ. Within a minute after the egg is deposited in water the capsule splits into two distinct concentric envelopes everywhere except about 1/25 of its area. The outer envelope then wrinkles and peels off, but the two envelopes remain fused together

in the unsplit area. As the outer envelope peels off it inverts and its original inner surface adheres to the substratum, thus forming a base and pedicel by which the egg is attached. In final appearance the egg and its envelopes somewhat resemble a wine cup: the former outer envelope is the enlarged base, the point where the capsule did not split is a short stem or pedicel, and the inner envelope is a closed cup around the egg. Due to the lack of adequate histological sections, the structure of the capsule before splitting could not be determined. Figure 62 shows the structure of the part of the capsule left around the egg about 24 hours after splitting. It shows a thick band containing four regular rows of dark-staining structures that I interpret to be degenerating nuclei. The band is separated from the egg by a much thinner band that appears to be not nucleated. Since the thick band is believed to have been nucleated and the narrow one not, it is probable that the former is derived from the ovary and the latter is a secretory product of the ooplasm.

Teleosts in general. --Although the eggs of the three species described above are fundamentally alike, there are important differences among their three main components. A few examples taken from the literature will suggest the wide extent of variation among the eggs of teleosts in general. The eggs of Serranus atrarius are pelagic (Wilson, 1891). Although most teleost eggs are spherical, Breder (1943) listed 92 species in which the eggs approximate the shape of a prolate ellipsoid. The size of the egg varies from a few tenths of a millimeter to 10 mm. or

more (Brachet, 1935). Indeed Hildebrand and Schroeder (1928) reported that the egg of Galeichthys felis measures 20 mm. in diameter. Table 6 gives the diameter of the eggs of some species mentioned in this paper.

TABLE 6

SIZE OF EGGS

Species	Egg Diameter in mm.	Source of Information
Serranus atrarius	1.0	Wilson (1891)
Artedius lateralis	1.07	Budd (1940)
Clinocottus analis	1.25	Budd (1940)
Fundulus heteroclitus	2.0	Hildebrand and Schroeder (1928)
Opsanus tau	5.0	Hildebrand and Schroeder (1928)
Salmo gairdneri	4.5-5.0	Original
Galeichthys felis	20.0	Hildebrand and Schroeder (1928)

Eggs show a wide range of colors, especially of the yolk. The yolk of eggs is cherry red in Artedius lateralis. tannish yellow in Clinocottus analis, brilliant golden yellow (Breder, 1943) in Bathygobius soporator and bright green or lavender in some other species (Budd, 1940). Among other differences, a few marine teleosts, such as the Embiotocidae, are truly viviparous and many others, including the Scorpaenidae, are ovoviviparous (Budd, 1940), but most species are oviparous.

This brief survey leads to the conclusion that very likely no two species have an identical combination of optically discernible morphological features. Therefore, it should be possible to build identification keys for eggs paralleling those for adults.

Holosteans (Amia calva). --In Amia the cytoplasm of the mature egg is white and forms a circular cap that is continuous with the yolk, which is charcoal gray. There is no oil droplet. The yolk consists of platelets of various sizes, distributed throughout the egg in a characteristic gradient. As in teleosts, the egg membrane closely adheres to the surface of the egg at stage 0.9, but its lifting is completed much later than in teleosts. It is inferred from Pierson's description that the egg membrane consists of an inner, non-cellular vitelline layer and an outer, cellular chorionic one that is provided externally with threads which are adhesive and remain so until the end of gastrulation. The egg is demersal and is slightly ovoid, being about 2.14 mm. in polar diameter and 2.16 mm. in equatorial diameter. Finally, the mature ovum of Amia is less telolecithal than the fertilized teleostean egg, and this may explain most of the morphological differences that occur in the early development of these two groups of fishes.

Fertilization

Lifting of the egg membrane. --Within a minute after milt comes into contact with the egg of the logperch, lacunae appear between the

ooplasm and the egg membrane, quickly merge and establish a perivitelline space between the egg and its membranes. This space rapidly widens, until within ten minutes it reaches an average width of about 0.2 mm. When the membrane starts to lift, the egg shrinks about 0.1 mm. in diameter, but when lifting is complete the egg has resumed its original volume and also has gained in transparency.

The zebrafish egg shrinks by roughly 12% of its original volume during the formation of the perivitelline space (Roosen-Runge, 1936, 1938). Okkelberg (1914) found the shrinkage in the brook lamprey eggs to be 14%. This shrinkage, together with the fact that the perivitelline fluid is more viscous than water, indicates that even though water is imbibed, the egg also contributes some material to the fluid. The subsequent restoration of volume and increase in transparency of the egg indicates that hydration compensates for the loss of fluid to the perivitelline space.

Pierson's (1953) observations on membrane-lifting in Amia show interesting differences between teleosts and holosteans. The Amia egg is not free to rotate within its membrane at the 16-cell stage and membrane lifting is completed only at the tailbud stage.

Origin of the blastodisc. --In the logperch at stage 0.9 the ooplasm is a thin layer around the yolk mass (Figure 35). At stage 1.6, the last stage before the egg membrane is completely lifted away from the ooplasm, ooplasmic movements resembling those in an amoeba were seen

to start and to continue in a polar direction that resulted in concentration of cytoplasm to form over the yolk a hemispherical cap, the blastodisc. However, histological sections show that a thin layer of cytoplasm remains around the yolk, fully continuous with the circular margin of the blastodisc (Fig. 35).

A small residue of ooplasm remains within the core of the yolk in the ripe unfertilized egg (stage 0.9) of the zebrafish, but not in the logperch. This residue somewhat modifies the pattern of ooplasmic movements in the zebrafish, where it eventually becomes incorporated into the blastodisc but not concurrently with that of the cortical zone. While the streaming of cortical ooplasm in the zebrafish seems to follow a pattern similar to that in the logperch, the movements of that within the yolk are more vigorous but intermittent, and they continue through the segmentation stages. Throughout these stages there are striking fluctuations in the shape of the blastodisc and yolk. For lack of a better analogy, the movements of the yolk mass can be compared to the last contractions of a dying heart. The movements of the yolk fluctuate with cleavage and their intensity diminishes from cleavage to cleavage. Comparisons between serial sections of completed cleavages show that the amount of cytoplasm left in the yolk is decreasing. Roosen-Runge (1936, 1938) made a detailed analysis of the movements boldly sketched here.

Morphological progress. --The most important morphological progress achieved during this phase can be recognized by comparing Figure 34 with Figure 35. It will be seen that the egg has changed from centrolecithal to telolecithal. At the end of the fertilization phase, the teleostean egg is more strongly telolecithal than that of Amia, and this gap in degree of animal-vegetal polarity is wide enough to orient the early cleavage patterns of Amia and teleosts along two different lines.

Although it is first in time, the lifting of the egg membrane is second in importance. Through this lifting and through the formation of the perivitelline fluid, the egg has become free to rotate in a fluid presumably more favorable to its development than pure water, and it is protected against its environment by a membrane which has changed from a soft and flexible to a relatively tough and resistant state. Fish culturists refer to this as water-hardening of the egg. In Amia the egg membrane becomes tough and resistant during the fertilization phase but the egg does not become completely free to rotate until the tailbud stage is reached.

Segmentation

Stratification of the blastodisc. --The blastodisc begins to stratify in the logperch at stage 2.5. The equatorial planes of the first four cleavages, stages 2.1 to 2.4, are morphologically vertical. As diagrammatically shown in Figures 36 and 37, the second cleavage plane is

perpendicular to the first, the third, shown in Figure 38, is perpendicular to the second and therefore parallel to the first, and the fourth, shown in Figure 41, is perpendicular to the third and therefore parallel to the second. In short, each of the first four cleavage planes is vertical and is perpendicular to the previous one. At stage 2.3 all cells still have their external wall on the margin of the blastodisc and all, therefore, are marginal cells; but at stage 2.4 the blastodisc has acquired a core of four medial cells. Since floors of all sixteen cells of stage 2.4 are adjacent to the yolk, the cells form a single stratum. The cleavage of the four medial cells at the end of stage 2.4 is in a horizontal plane, perpendicular to the polar axis of the egg, making a two stratum core of eight medial cells, with marginal and peripheral cells in a single stratum around it. From then on, the proportion of horizontal cleavages increases, extending both the width and the thickness of the stratified area through the entire blastodisc until at stage 2.9 it consists of nine to eleven strata of interlocked cells.

Method of segmentation. --In the three species studied here, segmentation is strictly confined to the blastodisc. In the logperch mitosis is synchronous in all cells of the blastodisc until stage 2.5 is reached, but sections of later stages show that several cells are out of the general mitotic pace. At 23°C., the average number of generations in two hours is five. Measurements taken on camera lucida drawings of sections show that the diameter of most daughter cells is about half that of their

parent cells but the size of the nucleus remains fairly constant. As will be reported in connection with the formation of the periblast, certain daughter cells do not repeat the cell wall pattern of their parent cells. Finally, the facts reported in the preceding paragraph indicate that the cells of each stage are oriented with respect to the base of the blastodisc in a definite plane peculiar to that stage.

Cleavage in the zebrafish is more synchronous. It has been followed to blastulation by Roosen-Runge (1938). As earlier mentioned, each cleavage is accompanied by movements toward the blastodisc of whatever ooplasm is still present within the yolk. Under similar environmental conditions, the cleavage rate in this species is twice that in the logperch.

These observations in both the logperch and zebrafish indicate that mitosis during segmentation differs in character from that usually seen in somatic cells. Mitoses during segmentation are almost synchronous, they follow each other in rapid succession, and the daughter cells do not grow to parent size, nor do they necessarily repeat the wall pattern of the parent cell. These characteristics, however, vary according with the species.

Periblast. --The separation of embryo from yolk in teleosts introduces problems regarding the availability of nutrients. These problems are solved by the embryo through its use of a periblast, a peculiar syncytium that surrounds the entire yolk mass. Even though the history

of this structure goes beyond the phase of development considered here, it will be brought to completion.

The primordium of the periblast in the logperch is the thin layer of cytoplasm left around the yolk between stages 1.6 and 1.9, when the egg changes from centrolecithal to telolecithal. This thin layer does not take part in mitosis. Although Wilson (1891) calls it an early marginal periblast, the expression "extraembryonic periblast" is more appropriate.

The embryonic periblast is established as a non-nuclear cytoplasmic layer in the following manner: at the end of the first cleavage, shown in Figure 36, the vertical separation between the resulting two cells does not extend entirely to the yolk. The floors of both cells extend horizontally laterad from the lower margin of this partition, but are never completed. Also, there remains beneath the floor of the two blastomeres a thin layer of cytoplasm, which is the forerunner of the embryonic periblast. The yolk mass is now completely surrounded by a distinct layer of cytoplasm that extends beneath the blastomeres and is continuous with the cytoplasm of each cell through the gap left between its floor and its lateral wall. A similar gap also is retained by all cells resulting from the second and third cleavages, as represented by Figures 37 to 39, but the four medial cells produced by the fourth cleavage lose it, thereby losing all connection with the periblast (Figures 41, 42). The periblast at stages 2.5 and 2.6 is not changed; it still is

a thin layer of cytoplasm surrounding the entire mass of yolk, and is continuous with the cytoplasm of marginal blastomeres.

The second or nucleating phase in the development of the periblast starts at stage 2.7, after some marginal cells, such as ma_1 of Figure 43, have cleaved horizontally and given rise to daughter cells with a pattern such as ma_1' and ma_1'', respectively. Ma_1' acquires the wall pattern of the parent blastomere, its floor remaining open laterally to the periblast, but ma_1'' forms no wall, its nucleus and cytoplasm being left in communication with the periblast. At stage 2.9 the ring of periblastic nuclei beneath the margin of the blastodisc is complete, and even the marginal cells, represented by x in Figure 45, have lost their cytoplasmic connections with the periblast.

The nucleation of the center of the embryonic periblast in the logperch is achieved during blastulation, through an initial immigration of nuclei (shown by z in Figure 46) from the medial cells adjacent to the periblast, and through subsequent nuclear divisions within the periblast itself. At stage 3.6 the embryonic periblast is an almost uniformly nucleated syncytium (Figure 47, e. pb.), although it has a greater concentration of nuclei in its thick margin (Figure 47, m. pb.). The margin thins out peripherally into the extraembryonic periblast, which becomes nucleated as the margin of the blastodisc spreads out around the yolk in an epibolic movement. The nuclei for the extraembryonic periblast are contributed through division of those in the marginal periblast

as it advances beneath the advancing germ ring. By the time the gastropore closes the embryonic and extraembryonic periblasts have reached their full development and are one hollow sphere encompassing the yolk and oil globule (Figure 48).

As the yolk diminishes in volume, the periblast disintegrates. Its last position is in the liver, as shown in Figure 49, where it encompasses the remains of the oil globule. All of both the periblast and the oil globule disappear four days after hatching, when the larva has reached the stage of feeding.

In the zebrafish the formation of the embryonic periblast occurs late. No uncleaved cytoplasm is left between the blastomeres and the yolk during early cleavages. The foundation of the embryonic periblast is not laid until stage 2.7, when the cytoplasm and nuclei of some marginal cells are continuous with the extraembryonic periblast, as shown in Figure 63. By the time stage 3.2 is reached, however, the embryonic periblast is uniformly nucleated, and its fate thereafter is similar to that in the logperch.

According to the description of Wilson (1899), the formation of the periblast in Serranus atrarius is similar to that described above for the logperch. However, in Serramus atrarius the medial cells do not contribute to the embryonic periblast. According to the description of Kopsch (1911) for Salmo trutta (as S. fario), no cytoplasmic layer is left in this species during early cleavages as a foundation of the embryonic

periblast, and the nuclei for the periblast are derived from the marginal and medial cells of the blastodisc. Therefore, the method of formation of the periblast in Salmo trutta is similar to that in the zebrafish.

Morphological progress. --The organism has progressed to a multicellular state, from one to more than five hundred cells. Its volume and shape however have remained nearly unchanged, but there has been an increase in the ratio of nuclear to cytoplasmic material. The foundation of both the embryo and the periblast have been laid, but the morphological symmetry is still radial.

Amia reaches the same degree of organization, but by somewhat different paths. Despite the mildly telolecithal structure of the egg of Amia, the first seven cleavages are holoblastic, an unusual procedure among telolecithal eggs and unknown among teleosts. After the seventh cleavage, the marginal cells do not complete their division furrows peripherally, and the result at the end of segmentation is an ovoid blastula consisting of a relatively small cap of micromeres that are in sharp contrast with the macromeres below. In this holostean the embryonic periblast is recognizable as a distinct layer before the extra-embryonic one appears, and the center of the embryonic periblast becomes nucleated before its margin is nucleated. This order of events in establishing the periblast is the reverse of that found among teleosts.

Blastulation

In the logperch. --In describing the main events of blastulation, morphologists usually put much emphasis on the formation of the blastocoele. The fact that it is absent in this species forces one to concentrate on the progress of other features. The changes in the geometrical pattern of the blastodisc will be described in relation to an imaginary plane passing horizontally through the margin of the blastodisc and termed the marginal plane. At stage 3.0 the blastodisc of the logperch is hemispherical and, as seen in Figure 26, its base coincides with the marginal plane. The peripheral cells are relatively low, appearing almost rectangular in cross section, but the medial cells are more nearly spherical, and they are more loosely arranged. Since there is no blastocoele, the lower cells of the blastodisc always lie on periblast, and this lies directly on the yolk.

As the blastodisc become more compact it loses height above the marginal plane, but it gains depth below that plane. It thereby acquires a biconvex form, but with the lower convexity less pronounced than the upper. This form, shown in Figures 27 and 57, is typical of stage 3.2. The changes in shape between stages 3.2 and 3.9 occur mainly in the floor of the blastodisc; the perimeter of the blastodisc increases without affecting its outer convexity. At stage 3.4 the floor has returned to the marginal plane, giving the blastodisc the plano-convex appearance shown in Figures 28 and 58. The floor continues to rise until at stage 3.6 it

arches above the marginal plane, producing the converging concavo-convex form shown in Figure 29. Although the top of the blastodisc remains convex throughout the blastular phase, the front half of the floor continues to rise and the rear half becomes slightly depressed by a greater thickening in this area, resulting in the eccentric concave base shown for stage 3.8 in Figures 30 and 60. At the end of the blastular phase, the blastodisc is composed of a peripheral stratum of cuboid cells, the periderm, and a central compact mass of morphologically indifferent polygonal cells. Their form resembles that of ordinary epithelial somatic cells, and mitosis is less frequent than in younger stages.

In the zebrafish and the smelt. --In the zebrafish blastulation follows a plan generally similar to that described for the logperch. It differs, however, in certain striking respects: a transient segmentation cavity, or blastocoele, appears during early cleavage stages, but is obliterated later in blastulation. The embryonic periblast is uniformly nucleated in an earlier stage, at 3.2 rather than at 3.6 as seen in the logperch, and the peripheral layer or periderm also is differentiated earlier, at stage 3.4, instead of at stage 3.8. In the smelt the periderm is differentiated at stage 3.2, and a larger blastocoele is formed and persists into gastrulation, as shown in Figures 64 and 65. The specimen shown in Figure 64 is unique among the twenty-five specimens of this species studied. It has a nucleated arm extending from the center of the embryonic periblast into the core of the yolk, a structure not found

in any other specimen of this species or in any other species.

Morphological progress. --In teleosts the relative position of cells is not greatly changed during blastulation. However the total blastodisc undergoes some morphological remodelling that will facilitate cell re-distribution along an antero-posterior axis during gastrulation.

In Amia the blastodisc at the end of blastulation is fairly similar in structure to that of teleosts, but, this final pattern is achieved in a different manner. The blastodisc becomes biconvex by adding to its floor of cells that are contributed by completion from the underlying syncytium. The blastodisc then assumes a concavo-convex form like that in teleosts, but it omits their stage 3.8. This stage is replaced by further thinning out of the blastodisc until it is a cap six or seven cells thick. The superficial layer is differentiated into a complete periderm, as in teleosts, and the well-marked segmentation cavity formed in Amia during early cleavages is obliterated during blastulation.

CHAPTER V

GASTRULATION

Preliminary Considerations

Concept of gastrulation. --The accurate determination of morphogenetic movements made by Vogt (1925, 1929) on amphibians constitutes the basis for understanding gastrulation among vertebrates. He devised a method of tagging cells in living embryos and of following their fate through development. The method consists of applying to the egg surface a fragment of agar-agar impregnated with vital stain (Nile blue, neutral red, etc.). The stain colors a limited group of cells but does not interfere with their normal development: it does not diffuse beyond the treated cells, and it lasts long enough to allow their subsequent movements to be traced. Systematic application of this procedure to the various cells or groups in the late blastula and gastrula makes it possible to identify presumptive areas on the blastula and to trace their paths through gastrulation. These results are then graphically presented in a series of views of the embryo at different stages: the presumptive areas are mapped and their movements are indicated by arrows.

The use of Vogt's technique on amphibians and other vertebrates led embryologists to define gastrulation in dynamic rather than static

terms (Pasteels, 1940; Needham, 1942; Rugh, 1948; Da Costa, 1948; Nelsen, 1953; Witschi, 1956; Balinski, 1960). For instance, Nelsen (1953) defined gastrulation as:

> The dynamic process during which the major, presumptive organ-forming areas of the blastula become rearranged and reorganized in a way which permits their conversion into the body plan of the particular species.

This concept of gastrulation is so well established that its implication can not be ignored in descriptive embryology. In any investigation of morphogenesis in a particular species, the movements must be assumed, if not demonstrated.

The idea of gastrular movements in teleosts goes back to Götte (1869), who discovered the process of involution in this group, but he explained it in terms of directional growth rather than movement of cells. Keibel's (1901) interpretation was much closer to the modern concept when he defined gastrulation as "the process by which the entodermal, mesodermal and notochordal cells find their way into the interior of the embryo."

Pasteels' fate maps. --The turning point in understanding of gastrulation in teleosts received its first modern impetus when Pasteels (1933, 1934, 1936) applied Vogt's technique to Salmo gairdneri (as S. irideus). Oppenheimer (1935a, 1935b, 1936b) soon followed with similar work on Fundulus heteroclitus. The fact that their independently conducted investigations resulted in similar findings strengthens their respective

statements that, with minor variations, the pattern for gastrulation is the same for all teleosts. It remains to be shown how their findings apply to the species reported in this paper. Pasteels' fate maps, reproduced here in Figures 50-55, provide a useful basis for comparison; they give a remarkably clear idea of the overlap of events at five different stages.

Figure 50 represents the presumptive areas on the surface of the blastula. In Figure 51 invagination begins; primordia of entoblast, anterior end of notochord and first somites have invaginated at the dorsal lip, and convergence has begun. Figure 52 shows that invagination is taking place around the full margin of the gastropore. The presumptive notochordal cells that still remain on the surface converge toward the median line. The median portion of the neurectoblast shows a slight longitudinal extension, followed by convergence of its lateral parts toward the median line. The direction of the arrows that are showing both on the surface and beneath at the margin indicates that the lateral mesoblast, once it has started to converge, continues to do so, both before and after it involutes.

At the next stage, Figure 53, convergence is the most striking movement in progress, but the arrows on the epiblast in Figures 52 and 53 indicate that also epiboly is taking place. Extension is more pronounced in the stage represented by Figure 53 than before, and it is definitely the dominant movement at the next stage, Figure 54, when

the median portions of both the invaginated and the surface materials are strongly extended caudad. At this time also the last superficial vestiges of the notochord are invaginating, those of the neurectoblast are converging on the axis, as is represented on the right side of Figure 54, and the invaginated mesoblast is moving toward the notochord, as is seen on the left side of the Figure. The same processes are continued in Figure 55: they stop only when all cells of the presumptive areas have reached their final destinations along the longitudinal axis of the embryo.

Pasteels' fate maps clearly depict the order of events in Salmo gairdneri. The fact that they are accompanied by pertinent drawings and descriptions from life facilitates comparison with similar stages in other species. The following description of gastrular stages in the logperch is illustrated by drawings of the blastodisc in Figures 31-33, of midsagittal sections in Figures 75-79, and of cross sections in Figures 80-84.

In the Logperch

Beginning of gastrulation. --Stage 3.9 marks the end of blastulation. The blastodisc of this stage is characterized by a rudimentary longitudinal axis consisting of optically indifferent cells in a solid mass of increasing thickness from anterior to posterior, as represented in Figures 75 and 80. The mass is uniformly convex externally, and the posterior region is somewhat convex beneath. The entire blastodisc is

covered with a simple cellular layer of well-differentiated periderm that meets the periblast in the full circle that forms the margin of the blastodisc.

About 1 1/2 hours after stage 3.9 is reached, the first evidence of involution that can be seen on the living egg appears. It is an opaque spot at the presumptive dorsal lip of the otherwise transparent rim of the blastodisc. This spot and the slight depression extending along the margin of the blastodisc on each side of it are the main distinguishing features, in life, between stages 3.9 and 4.0. Longitudinal sections through this opaque spot in stage 4.0, seen in Figure 76, show a rather short wedge of cells (i.c.m.) projecting beneath the blastodisc from its posterior margin. Such evidence of involution at this stage is not found at any other point in the margin of the blastodisc, as represented by the left side of Figure 76 and in Figure 81. When Figure 81 of stage 4.0 is compared with Figure 80 of stage 3.9, the axis is seen to have become more sharply delimited. This is the result of convergence of cells toward the median line. Evidence of epiboly is not easily detected: the total surface area of the blastodisc at stage 4.0 is not perceptibly greater than that at stage 3.9. When Figures 76 and 81 are compared to Figure 51 it is seen that the progress of gastrulation at stage 4.0 in the logperch is similar to that of the early gastrula in Salmo gairdneri. However, the blastodisc at this stage in the logperch covers fully one-third of the yolk, but in Salmo it covers less than one-sixth.

Formation of the embryonic shield. --Stage 4. 5 of the logperch, equivalent to the stage of Salmo represented in Figure 52, is reached three hours after stage 4. 0. Polar views of embryos of the logperch show an eccentric translucent area that corresponds in shape and location to the epiblast shown in Figure 53, from Pasteels. This area is bordered anteriorly and laterally by the light gray margin of the blastodisc, and posteriorly by a light gray plate.

Comparison of histological sections of stages 4. 0 and 4. 5 shows evidence of architectural changes. Midsagittal sections through ventral and dorsal lips show that the short wedge of cells projecting inward from the dorsal lip at stage 4. 0 has reached forward to the center of the blastodisc at stage 4. 5, as represented in Figure 77. There also is an inward projection of cells at the ventral lip in stage 4. 5, and sections through lateral lips of this stage 4. 5, shown in Figure 82, give evidence of involution there too. These points of involution in the sections that are a ledge, began at the dorsal lip and progressed as a crescent around the margin of the blastodisc to the lateral lips and finally met at the ventral lip.

Sections through the translucent area, mentioned above for the blastodisc of living logperch embryos, show that this area is composed of a simple layer of peridermal cells superficially and one or two layers of polygonal epiblastic cells beneath. The blastodisc, therefore, is

relatively thin in this crescentic area, which is extraembryonic. The embryonic area, or shield, is about as thick as it was in stage 4.0 but it now consists of both the original upper portion and an involuted lower portion.

Knowing these features, one can reconstruct the ar hitecture of the blastodisc. The blastodisc covers half of the yolk at stage 4.5. It still is dome shaped, but its thickness has become graduated and its margin is folded under to form a continuous rim. This fold is broadest and thickest at the rear, the dorsal lip. It has extended forward from the rear and inward from the sides to underlie most of the embryonic shield, but it does not underlie most of the extraembryonic area.

Interpreted in the light of Pasteeels' (1936) wo... these architectural changes are the result of epiboly of epiblast and involution and extension-convergence of the chordamesentoblast, as well as extension-convergence of the neurectoblast. Most of the entoblast and chordamesoblast have already involuted at the dorsal lip, and the lateral mesoblast is being involuted around the remainder of the blastodisc, whose entire margin is the equivalent of a gastropore. The non-involuted material of the blastodisc is a sheet of increasing thickness from anterior to posterior. It consists of a superficial differentiated periderm externally, and under this a thin layer of epiblast that is one or two cells thick in the extraembryonic area, and a thicker mass that is neurectoblast, but

also some mesoblast that has not yet involuted, in the embryonic area.

Differentiation of germ layers is now clearly in progress. Because the start of differentiation is a pertinent landmark, it is suggested that the stage in which it begins be designated as the middle gastrula, therefore stage 4.5. This also is the stage when the embryo has reached the form of a shield, an easily recognized and important intermediate achievement toward longitudinal axiation. The extent to which the blastodisc covers the yolk is here not an important consideration. That it covers half of the egg at this time is only coincidental and should not be taken as a criterion for identifying the middle gastrula of a teleost. Indeed, in Salmo this stage is reached before the blastodisc covers one-sixth of the egg.

Indentation of the embryonic shield. --Stage 4.7 in the logperch is comparable to that of Salmo shown in Figure 53. In Salmo the blastodisc then covers only one-sixth of the egg, but in the logperch it covers three-fourths. The embryonic area in both is still a shield, but its anterior margin is bilaterally indented and its anteroposterior axis is slightly longer than before, as shown in Figure 78. Figure 53 of Salmo shows that the bilateral indentation of the blastodisc is the result of extension-convergence. Cross sections through the extraembryonic area of stage 4.7 of the logperch, represented in Figure 83, show that this consists of an outer flat periderm and an inner layer of epithelium that are in contact with each other but are separated from the periblast by a

cavity. The amount of involuted material at the lateral and ventral lips is slight in comparison to that at the dorsal lip. Cross sections through the middle of the shield show a clearer stratification of neurectoblast and mesoblast but no appreciable difference in the shape of cells. The cells of the entoblast are low, and those of the other two germ layers are taller.

Formation of the embryonic keel. --The last recognizable event in gastrulation is the elongation of the embryonic shield, which is the embryo. When this is complete, in stage 4.9, the embryo has the length and compactness of a rod but is not entirely cylindrical: its undersurface is angular and dips into the yolk (Figure 69) like the keel of a ship into water. In surface view the embryo resembles a rod whose base is broadly connected to the germ ring. The rod and its base are light gray in color, but the rest of the blastodisc is translucent. This stage, 4.9, corresponds to Figure 54. However, Salmo reaches this stage when the blastodisc covers only one-third of the yolk, but the log-perch does not reach it until the blastodisc covers five-sixths of the yolk.

The main factors responsible for the transformation of the embryonic shield into a keel are the longitudinal extension of the median portion of the shield and the convergence of its lateral portions toward the median line. Figure 69 shows how the anterior region of the keel is formed by convergence of the neurectoblast, and Figure 66 shows that the mesoblast does not yet reach the median line this far forward. The

neurectoblast is thick medially and grades laterally into the thin epithelium that covers the yolk, and the mesoblast, which is three cells thick medially, tapers laterally to one cell thick and finally ends beneath the neurectoblast before the latter merges with the embryonic epithelium. Figure 70 shows that the neurectoblast is thinner midway along the keel than in the head region. As in stage 4.7, there is not much evidence in stage 4.9 of histological differentiation. However, the orientation of the cells allows recognition of the boundaries of the notochord medially, the mesoblast laterally of it, the ectoblast dorsally of them, and an irregular layer of entoblast ventrally of them (Figure 67). Figures 68 and 71 show no sign of keel formation in the caudal end of the embryo. At this level on the axis the only obvious differentiation into germ layers is laterally.

This short analysis of the structure of the stage 4.9 embryo shows that:

1. Convergence is more pronounced in neurectoblast than in chordamesoblast.

2. The keel forms from anterior to posterior along the axis.

3. The neurectoblast is more abundant in the head region that toward the rear of the embryonic axis.

4. Differentiation of notochord, mesoblast and entoblast is more advanced in the middle than toward either end of the axis.

These features are considered to mark the end of gastrulation.

Pasteels' intention of following morphogenetic movements to their completion fully justifies his inclusion of a later stage, Figure 55, in his study. The descriptive morphological approach to development differs from the experimental approach in that it considers development in terms of the major trends that characterize the several periods of development. When the stage represented in Figure 55 is reached, other major trends have overshadowed gastrular movements, and development therefore has passed beyond the gastrulation phase.

Periderm. --In the logperch the periderm is already differentiated at stage 3.8, when it is a continuous cuboidal epithelium over the entire blastodisc (Figs. 75, 80). The contrast in shape between these cells and those beneath them facilitates the task of finding whether they take part in morphogenetic movements. At stage 4.0 the peripheral cells of the periderm meet the marginal periblast but are not continuous at any point with the layer that is involuting at the margin of the blastodisc beneath themselves; they become even more crowded, and change from cuboidal to conical. This change in the shape of these cells suggests that they are dragged in by the involuting cells of the blastodisc and do not have any tendency to involute. The rest of the periderm continues to expand with the blastodisc as the latter proceeds to cover the yolk. The increase in the area of the periderm is accomplished through gradual collapse of its cells until they are hardly distinguishable from the blastodermal cells beneath. At the closure of the gastropore the periderm is

lifeless but still functional as a protective layer.

These observations on the periderm of the logperch, together with those on the zebrafish and smelt, confirm the findings of Götte (1873) and Pasteels (1936) for Salmo gairdneri, of Wilson (1891) for Serranus atrarius, and of Sumner (1903) for Salvelinus fontinalis.

In the Zebrafish and the Smelt

Morphogenetic movements in the zebrafish were demonstrated by means of time-lapse photomicrography by Lewis and Roosen-Runge (1944) and by Hisaoka, Ott and Marchese (1957). My serial sections reveal minor differences between the zebrafish and the logperch. The involuted material is more loosely arranged in the zebrafish than at comparable stages in the logperch. The progress of epiboly in relation to formation of the embryo appears to be the same in the two species up to stage 4.5. During stages 4.5 to 4.9, however, there seems to be an acceleration in keel formation in the zebrafish: the keel is complete when the blastodisc covers only three-fourths of the yolk in this species but not until it covers five-sixths in the logperch.

Similar differences occur in the smelt. At stage 4.5 its extraembryonic area is still two or three cells thick, and involution is like a proliferation of cells inward beneath the periphery of the blastodisc (Fig. 65). This resembles what Pierson described for Amia at her stage 13. The thickness of the extraembryonic area in the smelt is

reduced during the transformation of the embryonic shield into a keel, i.e. between stage 4.7 and 4.9. It does not reach stage 4.9, represented in Figures 72 to 74, until just before the gastropore closes.

Discussion

Heterochrony among gastrular movements.--Reference to the progress of epiboly in comparison to that of other gastrular movements has been made throughout the above description. The blastodisc covers about one-third of the yolk at stage 4.0 in the three species studied, but it covers only about one-sixth of the yolk at the same stage in Salmo. In all these species, however, the blastodisc eventually encompasses the entire yolk by epiboly. Meanwhile, the embryonic keel is forming. When the keel is completed, the blastodisc has engulfed three-quarters or more of the yolk in the three species I studied, but only one-third of it in Salmo. Therefore, epiboly lags behind other gastrular movements in all teleosts in which it has been reported, and especially in Salmo. When eggs are compared for size, those of Salmo are 5.0 mm. in diameter and those of the three species studied range between 1.0 mm. and 0.625 mm. in diameter. Pasteels (1936) feels that the delay in completion of epiboly in comparison to the progress of other gastrular movements is directly proportional to egg size. Among other facts he uses to support his statement, he includes a quotation from Clapp (1891), which states that in Opsanus the gastropore does not close until the

hatching stage, but he does not mention that its eggs are similar in size to those of Salmo gairdneri.

The degree of completion of epiboly in relation to other morphogenic processes among the three species I have studied gives little support to Pasteels' opinion. In the logperch egg, 1 mm. in diameter, the blastodisc covers five-sixths of the yolk at stage 4.9; in the smelt egg, 0.75 mm. in diameter, the blastodisc almost entirely covers the yolk at that stage; but in the egg of the zebrafish, which is only 0.625 mm. in diameter, the smallest of all, the blastodisc covers only three-fourths of the yolk at stage 4.9. It would be most interesting to know the degree of epiboly at stage 4.9 in Galeichthys felis, whose eggs, according to Hildebrand and Schroeder (1928), are very large. 20 mm. in diameter.

Thus synchrony between completion of epiboly with other gastrular movements varies among teleosts. Heterochrony appears greater among large eggs than small ones, but egg size is not the only factor that influences it. Even though the known facts suggest some sort of controlling mechanism over gastrulation in teleosts, it does not achieve a perfect synchrony.

Movements of the several germ layers. --The descriptions of the gastrular stages in the three species I studied are intended to give an idea of the order of events, and the differences among them necessarily

are stressed. If, however, the events concerning each germ layer are considered together, the fundamental similarities among the three species become obvious.

Axiation of entoblast, notochord and somites is achieved by movements that are similar in all three species. Involution is greatest at the dorsal lip of the gastropore from where the involuted cells fan out beneath the blastodisc, and by extension-convergence take their respective positions in and alongside the anteroposterior axis of the embryo. The path followed by lateral mesoblast, however, is different from that of entoblast, notochord and somites. Its cells involute not at the dorsal lip but at the lateral and ventral lips, that is at the lateral and anterior portions of the margin of the blastodisc. As the margin converges toward the caudal end of the embryo during the last half of epiboly, most of the cells that involute from it become lateral mesoblast, and the remainder merge into a caudal mass which contributes to a number of derivatives in the postanal region.

The material for neurectoblast does not involute. In the late blastula its cells form a thin crescent whose horns point anteriorly, toward the ventral lip. Extension elongates the crescent medially and convergence shortens its horns. This combination of extension along the median axis and convergence of its lateral portions eventually produces the columnar shape of the neurectoblast over the embryonic axis.

The gastrulative movement of the material destined to become epiblast does not resemble the movements that result in axiation of entoblast, chordamesoblast and neurectoblast. The epiblast must compensate for loss of the surface cells involuted to the interior and provide for the increase in area needed by the expanding blastodisc to envelop the yolk. These are accomplished by epiboly, which Pasteels (1936) states is by cellular movement and not cellular proliferation. His conclusion is based on three kinds of evidence: 1) The number of epiblastic layers decreases as gastrulation progresses, 2) The epiblastic cells are stretched gradually and converted into flat epithelium, 3) The number of mitoses in epiblast decreases as gastrulation progresses.

Teleosts and holosteans (Amia calva). --Comparisons between Amia calva and teleosts will be made insofar as possible from the descriptions and figures presented by Pierson (1953). It is pertinent here to survey her section on gastrulation for which she describes 10 stages, grouped as follows:

Early gastrula (stage 13),
Middle gastrula (stage 14),
Late gastrula (stages 15 to 22).

Pierson supported her observations by photographs of all 10 stages and by drawings of 5 preserved eggs, and followed her descriptions by a discussion of debatable points. The explanations for these photographs

and drawings are minimal, only the stage number and the visual angle being given. Four drawings of histological sections are given; they represent only the ventral lip of stage 13, the dorsal lip of stages 13 and 14, and the embryonic area of stage 14, and some of the structures shown are not labelled. The explanations for these figures are only statements of the views represented.

She summarized her stage descriptions in what she termed their "approximate temporal order," as follows:

1. Separation of the blastoderm into a thin superficial and a thicker deep layer, the future superficial and nervous layers of the ectoderm, respectively.

2. Migration of cells, primarily those of the deep layer, toward the mid-region of the future embryonic area, toward the periphery of the blastodisc, and toward the vicinity of the dorsal lip, with the resultant thinning of the antero-ventral area of the blastodisc.

3. Slight invagination of the superficial layer deep to the dorsal lip region, separating it from the yolk ventrally, and proliferation from this layer anteriad, over the yolk, of entodermal cells and cells of the marginal periblast. Proliferation is continuous and maximal at the dorsal lip region and is intermittent around the remainder of the margin of the blastodisc.

4. Involution of the deep layer at the dorsal lip to form chorda-mesoderm.

5. Progressive but spatially and temporally intermittent involution of the deep layer, peristomial mesoderm, around the entire margin of the blastodisc. In the early stage this appears more like proliferation than actual involution. --

6. Continuous epibolic growth of the blastodisc over the yolk, grading from maximal at the ventral lip to little or none at the dorsal lip.

7. Increasing proliferation of entodermal and periblast cells around the margin of the blastodisc with intermittent slight invagination, possibly correlated with the cleavage planes in the yolk region.

8. Posterodorsad migration of the peripheral cells of the deep layer of the ectoderm toward the median and anterior embryonic area, and the resulting absence of this layer in the anteroventral area.

9. Increase in size of the dorsal lip and lifting of that region of the blastodisc from the yolk below, with resultant enlarging of the primary cavity, or blastocoele, deep to the chordamesoderm.

10. Formation of the subgerminal or secondary cavity by adherence of proliferated entodermal cells to the ventral surface of the chrodamesoderm just anterior to the dorsal lip.

11. Closure of the blastopore, the yolk plug area peripheral to the border of the blastodisc, by a fusion over the yolk plug of the periblast cells which are continuous with the superficial layer of the ectoderm encircling the yolk plug. The cells of the deep layer of the ectoderm in the ventral lips of the blastopore move dorsad, joining and enlarging the tailbud.

12. The superficial and deep layers of the ectoderm at the posterior border of the original dorsal lip become indistinguishable from each other at the time of closure of the blastopore.

Let us first try to establish the types of morphogenetic movements in Amia. Pierson's events 3, 4, 5 and 7 refer specifically to involution, 6 deals with epiboly, and 8 can be interpreted as convergence. Throughout her section on gastrulation, Pierson makes extensive use of the term "proliferation." In the context it seems sometimes to refer to multiplication of cells only, and sometimes to both multiplication and subsequent migration of cells in a definite direction. Among the ten textbooks of embryology I have consulted, only Brachet (1935) and Balinsky (1960) define the term "proliferation," and both contrast it

with cell migration. To Dorland (1957) proliferation is a "reproduction or multiplication of similar forms, especially of cells or cysts." On the other hand, Pierson does not use the terms "convergence" and "extension." One or both of these movements may be implied in her usage of the term "proliferation," or she may have used this term to indicate a movement replacing convergence and extension in Amia. For lack of better evidence, Pasteels' (1940) opinion can be considered. He feels that even though Vogt's method has never been applied to the ganoids, the same morphogenetic movements that occur in other vertebrates occur also in them. Therefore I conclude that the proliferation described by Pierson also includes one or both of the movements listed for teleosts.

Let us now compare the movements of each germ layer in teleosts and Amia. In teleosts the axiation of chordamesoblast is achieved by involution mainly at the dorsal lip, and by subsequent convergence and extension. The chordamesoblast in Amia undergoes involution as in teleosts, at the same point but its position on the late blastula is not the same. In Amia the blastodisc at late blastula is separated into two layers (event 1), the deeper being chordamesoblast. After involution the chordamesoblast "proliferates anteriad." From then on I assume its movements to be similar to those found in teleosts.

The neurectoblast of teleosts is axiated by convergence of the lateral material toward the embryonic axis and by extension of the

median area over the same axis. Event 8 of Amia is a description of convergence. It can be inferred that this event results in the formation of a plate that later transforms into "a solid neural rod." This probably is the result of extension and consequently would make the pattern of axiation in Amia similar to that in teleosts.

In teleosts the entoblast of the late blastula is concentrated at the dorsal lip and is axiated by movements similar to those of chordamesoblast. Events 3 and 7 indicate that in Amia the entoblast of the late blastula is found at the entire margin of the blastodisc, but only in its superficial layer. Entoblast in Amia involutes not only at the dorsal lip but also around the entire gastropore, and event 7 indicates that the macromeres possibly contribute cells to this layer. I infer that: 1) The entoblast that is involuted at the dorsal lip of Amia subsequently follows the same path as in teleosts. 2) The entoblast that is involuted at other points follows a path similar to that of lateral mesoblast in teleosts, 3) The additional cells contributed by the macromeres in Amia may go either way, depending upon their original location in relation to the embryonic axis.

Although the behavior of epiblast in Amia is not mentioned by Pierson, it can be inferred from event 6 that it is similar to that in teleosts. As in teleosts, epiboly in Amia probably is by cellular movement and not by cellular proliferation Finally, the periderm of teleosts does not involute. Pierson's figure 15 shows that in Amia this stratum

involutes at the dorsal lip and not at the dorsolateral lip (Pierson's Figure 16). More evidence is needed on this point. If this and the other minor differences mentioned above are ignored, there seems to be a fundamental similarity of the respective movements of the germ layers in teleosts and Amia.

Let us now compare the order of events during gastrulation in teleosts and Amia. Pierson apparently meant in her list to locate the beginning of 12 different events in time, but not to indicate the overlapping nor the final results of these events. She does not refer to stages in this list, and her description of stages contains no reference to the list of events. It is necessary therefore to rely on her description of stages to correlate the events.

The location of the presumptive areas on the late blastula of Amia would differ from that in teleosts to the extent mentioned above. Involution begins at stage 4.0 in teleosts and is limited to the dorsal lip, where entoblast and chordamesoblast involute successively. Pierson's description of stage 13 shows that in Amia the two layers involute simultaneously: "The dorsal lip of the gastropore is represented by a thickened proliferated portion of the deep layer and a proliferated portion of the superficial one, both distinctly defined." This quotation is from her description of stage 13, which she listed as a late cleavage stage, but the same stage again is listed as an early gastrula where, among descriptions of other characteristics, she says: "This proliferation

of both the superficial and the deep layers occurs intermittently around the entire rim of the blastodisc, maximal at the dorsal lip and minimal at the ventral lip." Evidently a stage for Pierson is not necessarily a state of development at a given instant. Her late cleavage stage 13 in Amia corresponds to my stage 4.0 in teleosts, and her early gastrula stage 13 shows a character typical of my stage 4.5 in teleosts. At any rate, the two events seem to take place successively as in teleosts. Still in stage 13, Pierson describes differentiation between the embryonic and extraembryonic areas and therefore the formation of an embryonic plate such as occurs in teleosts at stage 4.5. She also states that the area of the blastodisc has increased until it now covers half of the original egg. It must be concluded that Pierson interprets her gastrulation stage 13 in Amia to encompass a range of development broad enough to include those features shown by Pasteels in both Figures 51 and 52. Differences between teleosts and Amia at these instants would result from differences in the location of the presumptive areas on their late blastula.

At stage 4.7 in teleosts the processes of stage 4.5 are continued without appreciably changing the general appearance of the embryonic area from its shield-like form, but the germ layers are more clearly established. Pierson gives corresponding characteristics for Amia at stage 14: "The three primary germ layers are now clearly established. The cap now covers approximately 230 degrees of the original egg." The

order of events here is probably similar to that represented for teleosts in Figure 53.

In teleosts stage 4.9 is characterized by the transformation of the embryonic shield into a structure with a longitudinal axis recognizably longer than the transverse one. This seems to be reached at Pierson's stage 15 in Amia: "The median embryonic thickening, in the form of a solid neural cord, is relatively constant in width throughout this time . . . this establishes the area of the embryo proper." The order of events between stage 4.7 and 4.9 can be assumed to be similar in Amia to that in teleosts and can be seen by comparing Figures 53 with 54.

Events between the formation of the embryonic axis and the closure of the gastropore are considered to take place after gastrulation; therefore stages 16-22 described by Pierson will not be discussed here. In continuing her description to the closure of the gastropore, however, Pierson follows the tradition established by Beard (1899) and Eycleshymer (1903) in their work on Lepisosteus osseus. In other words, the morphogenetic movements are followed to completion. As in teleosts, their heterochrony in holosteans reduces the value of this approach in comparative studies. For descriptive purposes therefore, development should be divided into phases and each phase should be characterized by a major trend in the progress of the embryo toward the adult form. A phase should be considered as ended when another major trend

appears. In holosteans, as in teleosts, the moulding of the germ layers into organ primordia is another major trend, and it begins before the germ layers are completely axiated. The first evidence of this moulding appears in the neurectoblast and, therefore, the start of neurulation should be taken to mark the end of gastrulation and the beginning of the next phase in both groups of fishes.

In connection with the heterochrony between epiboly and other movements, it is interesting to note that the condition in Amia is quite similar to that in teleosts with eggs of a diameter only one half or one third that of the Amia egg. At the time of closure of the gastropore Amia is at the two-somite stage but the zebrafish, Brachydanio rerio, is at the four-somite stage. The diameter of their eggs are 2.16 mm. and 0.625 mm., respectively. If heterochrony increases with egg size, it would be expected that the gastropore would close in Amia at a stage somewhere between that in Salmo and in the zebrafish, which is at about 20 somites. The decrease in heterochrony in Amia might be explained by the fact that the yolk is not completely inert: the macromeres might act as an organizer on the epiblast and accelerate its epibolic movement.

This lengthy comparative discussion of gastrulation in Amia and teleosts is justified by the objectives of this report, which are to correlate the tremendous amount of work done on fish embryology and reduce this information to order. Pierson's section on gastrulation was used to point out some of the difficulties encountered.

SUMMARY AND CONCLUSIONS

1. The purpose of this report is to integrate the particulars of the early embryology of teleosts. For its foundation I have sought out the basic features of early development in one form, the northern logperch (Percina caprodes semifasciata). This search was guided by concepts considered fundamental in general embryology and by comparison with available material on the zebrafish (Brachydanio rerio) and the American smelt (Osmerus mordax), and is supplemented by facts available in the literature on these and other teleosts and in the holostean Amia calva.

2. The following techniques were devised and employed:

a. The Russian method of securing the fertilization of eggs was modified and used. The testes of one male are macerated in a moistened Syracuse dish and about 60 eggs are stripped into it, where they are kept for 15 minutes and then washed and transferred to culture dishes. Applied to the logperch, this method yielded slightly more than 50% of fertilized eggs.

b. A method for controlling the breeding activities of the zebrafish in captivity. It consists of artificially holding breeding populations in darkness on the day and until the time fertilized eggs are wanted.

c. A method for fertilization and incubation of smelt eggs. This method included the placing of ripe males and females together in plastic bags where they immediately discharge spawn. The fish are then removed and the spawn is diluted. After one hour in diluted milt, the eggs lose their adhesive properties. The diluted milt is then washed out and enough stream water to partially submerge the eggs is poured into the plastic bags. These are inflated with oxygen, sealed and incubated at 45° F. This method was used only once and needs further testing.

d. A method for fixing and decapsulating eggs during early stages. Eggs are hardened in hot (40°-50°C.) Bouin's for four minutes, decapsulated, and returned to hot Bouin's for 10 minutes of fixing.

3. The existing systems of staging early embryos are of four types: numerical, chronological, nominal, and short stage-description. Analysis leads to the conclusion that these systems are subjective and are so vague, inconsistent and misleading as to be of little comparative use. It is proposed, therefore, to divide development into major units called phases and to consider as stages the successive recognizable landmarks by which the amount of significant progress achieved by the embryo during a given phase of development may be estimated. Characteristics for 21 stages are given. The detectability of these characteristics is reported for three species and their reliability in teleosts is confirmed from the reports of other investigators.

To express progress of development in a standardized manner,

it also is proposed to establish a numerical system of staging whereby whole numbers express phases and decimals express stages of progress. In this system, zero designates the pre-fertilization phase, one the fertilization phase, two the segmentation phase, three the blastulation phase, and four the gastrulation phase. The stages, subdivisions of phases are designated by decimal numbers after their phase numbers. Later stages within a phase are assigned higher numerals than earlier ones. The gaps between successive recognized stages are indicated by gaps in the numerical series. Finer staging is indicated by second order decimals; for instance, a stage midway between 1.5 and 1.6 becomes 1.55.

4. The description of early development is based on the morphological progress achieved in each phase and in each stage.

a. The mature ovum is in the pre-fertilization phase, and is described according to its three main components: ooplasm, deutoplasm and external membranes. The extent of variation in these components is illustrated by description of the three species studied and by facts drawn from the literature. The main characteristic of the mature ovum is that it is centrolecithal.

b. The most striking event achieved during fertilization phase is the change of the egg from centrolecithal to telolecithal. Although the first event in time is the lifting of the egg membrane, it is second in importance. The lifting of the membrane and the formation of the perivitelline fluid enables the egg to occupy and rotate in a fluid environment that presumably is more favorable

to it than pure water. The egg is protected against the more distant environment by a membrane that has changed from soft and flexible to relatively tough and resistant.

c. During the segmentation phase the blastodisc progresses toward a stratified and multicellular condition through a series of specialized mitoses. Mitotic divisions during segmentation differ from those of ordinary somatic cells in proceeding in rapid sequence and in being almost synchronous in most cells of the blastodisc. Furthermore, the daughter cells do not grow to parent size and not all repeat the wall pattern of the parent cell. The foundation for the periblast also is laid during segmentation.

d. During the blastulation phase the blastodisc is remodelled into a shape that facilitates cell redistribution along an antero-posterior axis. In this remodelling it assumes a sequence of common geometrical shapes that are used as landmarks for describing the continuous change. At the end of blastulation the blastodisc is convex with an eccentric concave base, and is composed of a single stratum of cuboid cells under which is a compact mass of morphologically indifferent polygonal cells. There is evidence that in many teleosts the blastocoele is absent or obscure.

e. Gastrulation is described according to the dynamic processes of this phase. Morphogenetic movements are assumed, and reference is made to Pasteels' (1940) fate maps for understanding how the embryonic shield is formed within the blastodisc, how this shield becomes bilaterally indented anteriorly and how it then is transformed into an embryonic "keel."

f. The main gastrular variable among teleosts seems to be the degree of completeness achieved by epiboly in chronological relation to other gastrular movements. Epiboly shows a tendency

to lag behind other movements in large eggs, but egg size is not the only factor. Even though some sort of controlling mechanism can be postulated, it does not achieve a perfect synchronization of all gastrular movements in teleosts.

g. Comparisons with salient features in Amia calva, as described by Pierson (1953), are added whenever they are pertinent. Her description of gastrulation is used to emphasize some of the difficulties encountered in interpreting the literature on the embryology of fishes.

APPENDIX

This work led me to search for some striking sexually dimorphic character for sexing logperch in the field. The deeper colors and the larger anal fin of the males (Reighard, 1913) are not convenient criteria for this purpose. Examination of these and other sexual characteristics of the first 54 specimens captured revealed that the midventral strip from the tip of pelvic fins to anus is devoid of scales in females but is covered by a single row of many-pointed, very large scales in males. This convenient and clear characteristic feature thereafter, was successfully used to separate the sexes in the two-thousand specimens handled during the breeding season of the years 1953-1956. The difference is so clearly marked as to be perceived visually or by touch.

Trautman (1957) and Hubbs and Lagler (1958) mentioned the presence of a midventral row of modified scales in the logperch, but are not specific on the possible dimorphism of this character in this species. Bailey (1956) states that one of the characters for identifying the genus Percina is that the midline of the belly is usually covered with scales, at least in front of the anus. No further confirmation for my observations was found in the literature.

Examination of 34 males selected at random revealed that in all of them the row of modified scales was present and continuous posteriorly

and that in 17 it forked bilaterally beside the anus, as shown in Figure 8. The scales in the row were enlarged and strongly ctenoid in 31 and enlarged and without developed ctenii in the other three of the 34 specimens examined. My observations did not reveal discontinuities in the posterior extent of the series, as figured by Legendre (1952), see Figure 9 here. The forking of the series at the posterior end to flank the anus on each side (Figure 8), observed in half of the males examined, was not illustrated by Hubbs and Lagler (1958), see Figure 5 here, or by Legendre (1952), see Figure 9.

Winn (1958) concluded that among the fourteen darters he studied, the degree of versatility in reproductive behavior is directly related to the degree of evolutionary primitiveness. He also concluded that Percina caprodes is one of the most versatile species in the group. Among other supporting facts Winn mentioned (p. 72) a lack of dimorphism in this species. This statement may be questioned on the ground that Winn overlooked the presence in males and absence in females of modified scales on the midventral line of the belly.

New evidence for the versatility of the logperch is given by my observations on populations presumed to be descendants of those studied by Reighard (1913). The spawning sites he described were the sandy shoals of Douglas Lake, more especially those of South Fishtail Bay. Figures 2 and 3 are photographs of logperch spawning in this area, some 40 years after Reighard's observations were made. This spawning site

is along a sunny, sometimes heavily-wave-beaten, sandy shore, in water less than nine inches deep.

This species was observed to use a strikingly different spawning site nearby during the breeding season of the years 1951 to 1954. The site preferred in those years was a man-made harbor at the Biological Station. The harbor is about 40 feet square and is connected with the lake by a trench, about 10 feet wide and 60 feet long. The harbor was enclosed in a boathouse and was well protected against sun and waves, the water was about three feet deep, and the natural sand bottom was well covered with detritus. Logperch spawned there in greater abundance than on the kind of site described by Reighard. At the peak of the breeding season in 1952 an estimated 500 individuals were observed in the harbor, as compared to only three or four schools of about 50 each along 1, 000 feet of the shore. The boathouse has not been connected to the lake and the harbor has not existed since 1955; consequently the population has returned to its previous spawning ground along the shore.

The successive use of the two strikingly different spawning sites by this genetically continuous population seems to be unique, at least among the darters studied by Winn, and therefore adds more evidence to the versatility of Percina caprodes.

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PLATE I

SPAWNING SITES OF PERCINA CAPRODES AND OSMERUS MORDAX

Figure

1. Percina caprodes, at rest on the bottom, anterior part of the body supported on the deflected pectoral fins. Approximately natural size.
2. Photograph of Percina caprodes spawning in 1954 on the same shoal as described by Reighard in 1913.
3. Close-up view of spawning on the shoal.
4. Meach Lake and tributary stream (Gatineau Cty, P.Q., Canada). The stream is 200 feet long, and is about 3 to 6 feet wide and 12 inches deep in the spawining season. Omerus mordax has used it for spawning since about 1929.

PLATE I



Fig. 1



Fig. 2



Fig. 3



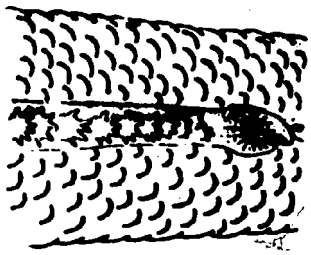
Fig. 4

PLATE II

Figure

5. View of the anal region of Percina caprodes (after Hubbs and Lagler, 1958).
6. Left preopercle of Percina caprodes, showing smooth ventral and posterior margins.
7. Breeding aquarium for Brachydanio rerio. On the left is the sand shelf and on the right is the breeding area covered with marbles.
8. View similar to Figure 5, but showing the forking of the row of modified scales at the anus.
9. Same view as Figures 5 and 8 (after Legendre, 1952).
10. Screening apparatus for eggs of Brachydanio. A, lower end of siphon. B, detritus screen. C, funnel. D, egg screen. E, funnel.

PLATE II



Midline of belly with a definite single file of enlarged, caducous scales which are separated by a slight groove from the scales on either side (northern logperch, *Percina caprodes semifasciata*).

Fig. 5

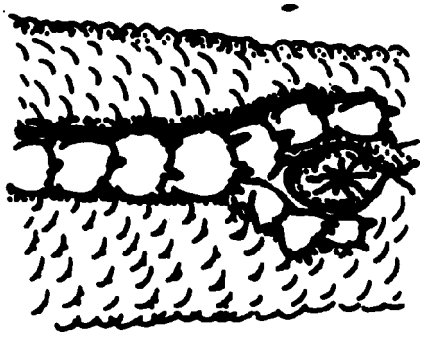


Fig. 8

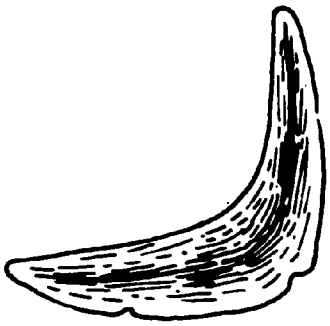


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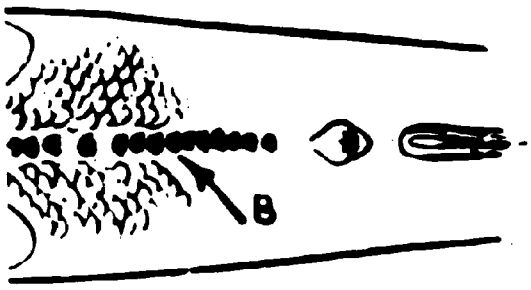


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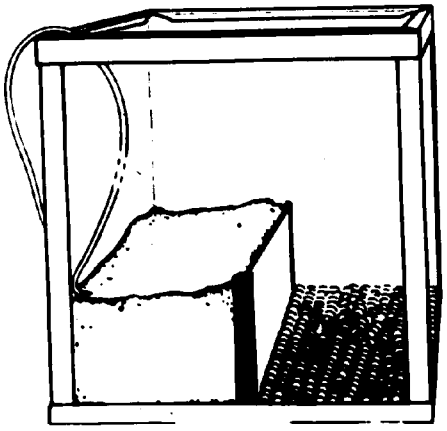


Fig. 7

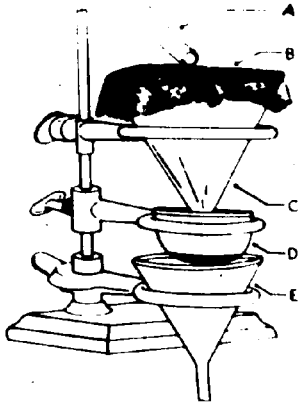


Fig. 10

PLATE III

Figure

11. Siphoning of Brachydanio rerio eggs from between the marbles.
12. Yolk of Osmerus mordax, x 400. Compare with Figures 14, 59.
13. Egg membrane of Percina caprodes before fertilization, x 1250. Compare with Figure 61.
14. Yolk of Brachydanio rerio, x 400. Compare with Figures 12, 59.
15. Mechanized microtome, developed by Dr. Q. N. LaHam (Anonymous, 1955).

PLATE III

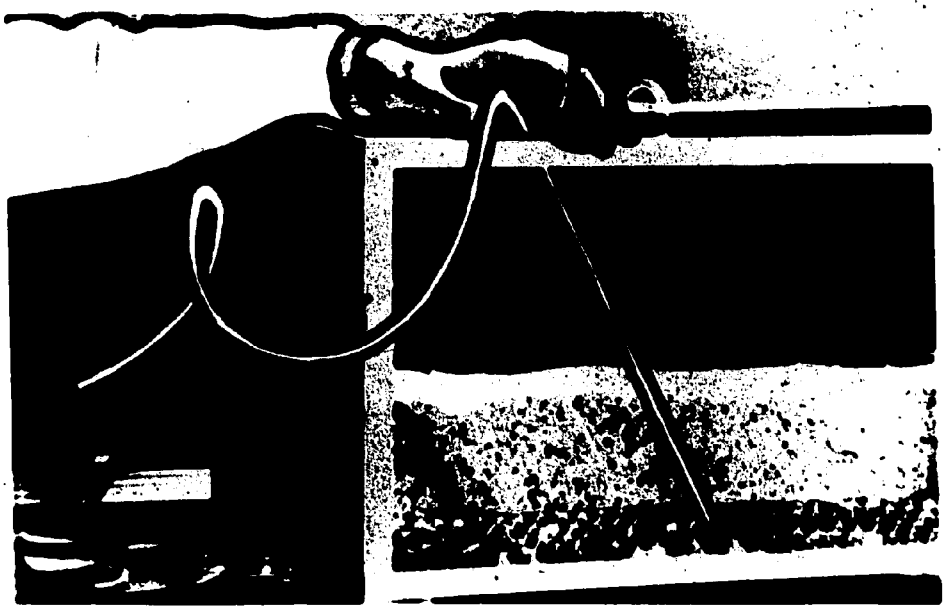


Fig. 11

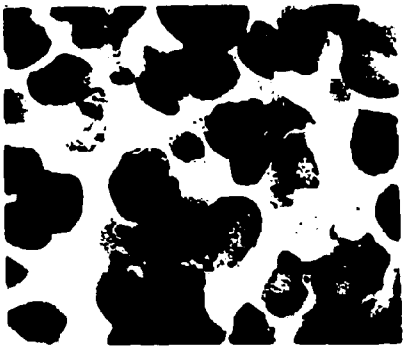


Fig. 12

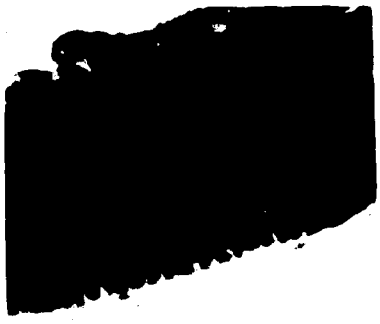
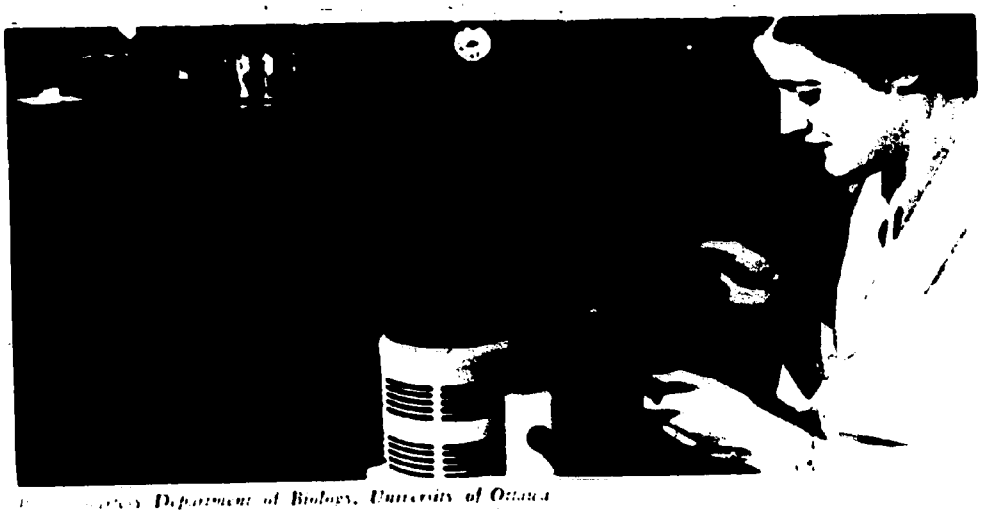


Fig. 13



Fig. 14



Department of Biology, University of Ottawa

Fig. 15

PLATE IV

DEVELOPMENTAL STAGES OF PERCINA CAPRODES

(Ages are averages taken on one hundred eggs)

Figures

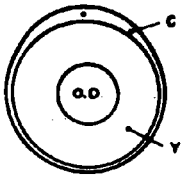
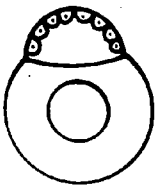
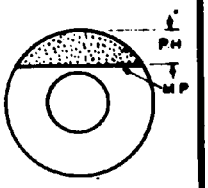
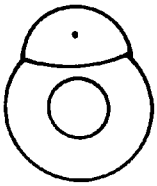
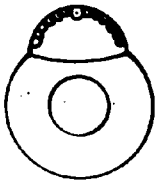
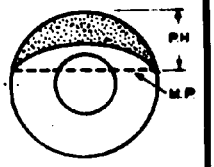
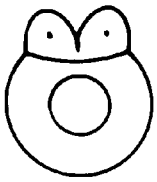
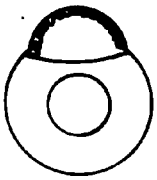
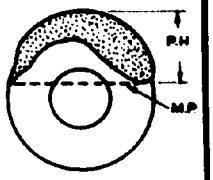
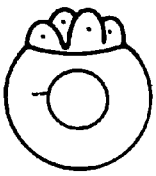
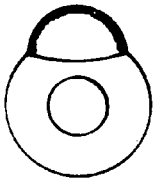
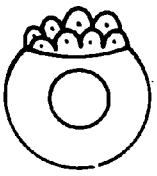
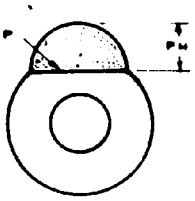
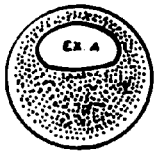
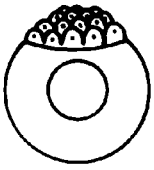
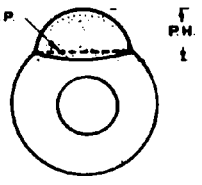
16-30. Blastodiscs, lateral views.

31-32. Blastodiscs, polar views.

33. Embryo, dorsal view.

C. cytoplasm
O. D., oil droplet
M. P., marginal plane
P. H., polar height
Y, yolk

PLATE IV

STAGE NUMBER	AGE-HOURS 20°-23°C		STAGE NUMBER	AGE-HOURS 20°-23°C		STAGE NUMBER	AGE-HOURS 20°-23°C	
0.9	0		2.5	3½		3.4	10¼	
1.9	1		2.6	4		3.6	11¾	
2.1	1½		2.7	4½		3.8	13	
2.2	2		2.8	5		4.0	14½	 POLAR VIEW
2.3	2½		3.0	7		4.5	17½	 POLAR VIEW
2.4	3		3.2	8		4.9	19½	 DORSAL VIEW

FIGURES 16–21 FIGURES 22–27 FIGURES 28–33

PLATE V

THE PERIBLAST OF PERCINA CAPRODES

Figure

34. Section through an unfertilized egg showing the cytoplasm (c.) evenly distributed over the surface of the yolk mass (y.). The cytoplasm is more abundant around the nucleus (n.).

35. Stage 1.9 in vertical section, showing the extraembryonic periblast (ex. pb.) connected to the blastodisc (bl.).

36. Stage 2.1 in vertical section, showing the incomplete floors (i.c.f.) of the cells and the thin layer of protoplasm constituting the embryonic periblast (e. pb.) left beneath them.

37. Polar view showing the plane of the first and second cleavages. Sections would show that the cells have incomplete floors similar to those in Figure 36.

38. Polar view showing the plane of the first three cleavages.

39. Section through plane CD of Figure 38. In this plane cell T_1 has a complete floor, but a section through plane AB would show that its floor is incomplete, similar to that of Figure 36.

40. Polar view showing the planes of the first four cleavages.

41. Polar view of stage 2.4. The blastodisc has a core of 4 medial cells (me.) surrounded by 12 marginal cells (ma.).

PLATE V

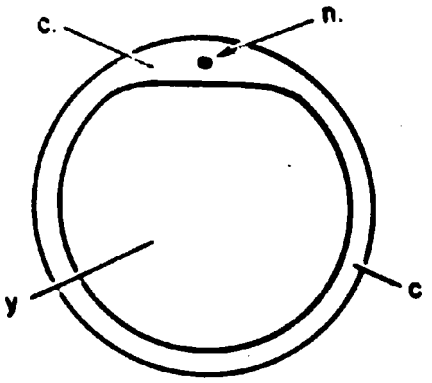


Fig. 34

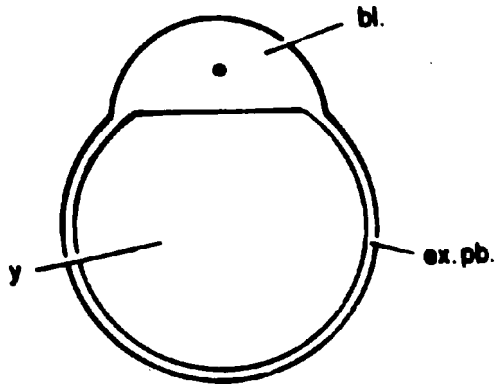


Fig. 35

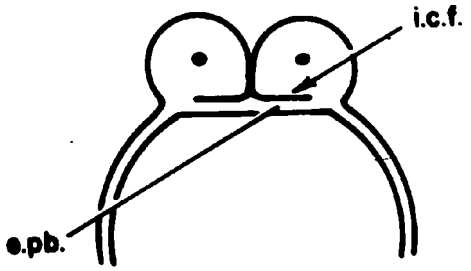


Fig. 36

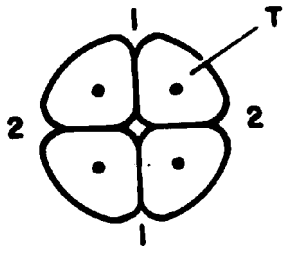


Fig. 37

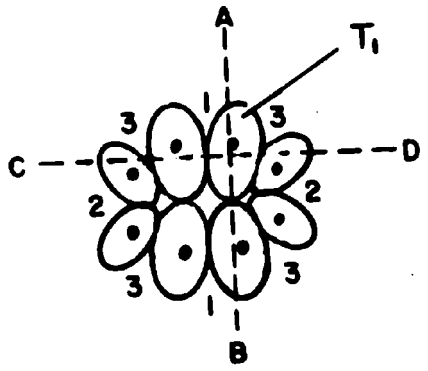


Fig. 38

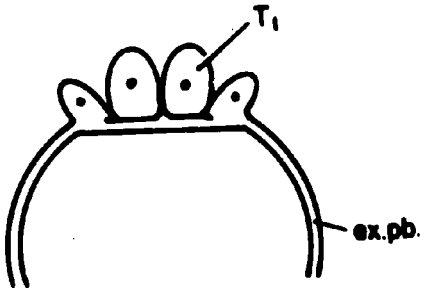


Fig. 39

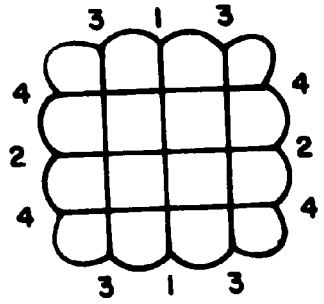


Fig. 40

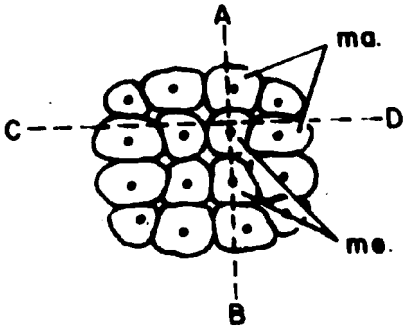


Fig. 41

PLATE VI

THE PERIBLAST OF PERCINA CAPRODES (Continued)

Figure

42. Stage 2.4 in section through plane AB or CD of Figure 41. Medial cells (me.) have complete floors and marginal cells (ma.) incomplete ones.

43. Stage 2.5 in vertical section. The medial cells (me.) of Figure 41 have cleaved in a plane parallel to the embryonic periblast, and the upper daughter cell (me_2) is not in position to contribute to the periblast. The marginal cells (ma.) of Figure 41, have cleaved in a plane perpendicular to the periblast but only the outer daughter cell (ma_1) of each has an incomplete floor.

44. Stage 2.7 in midsagittal section. A marginal cell of the blastodisc has cleaved in a plane parallel to the embryonic periblast and its daughter cell (ma_1'') has no wall while the other (ma_1) has an incomplete floor similar to ma_1 of Figure 43. Both the nucleus and cytoplasm of ma_1'' are integrated into the periblast.

45. Stage 2.9 in midsagittal section. A marginal cell of the blastodisc has cleaved in a plane parallel to the periblast, and daughter cell (x) has no cytoplasmic connection with the periblast but daughter cell (y) is integrated into the periblast.

46. Stage 3.4 in midsagittal section, showing the contribution (z) of the medial cells to the periblast.

47. Stage 3.6 in midsagittal section. Nuclei are distributed in the embryonic periblast (e. pb.) but not in the extraembryonic periblast (ex. pb.). There is a concentration of nuclei in the marginal periblast (m. pb.).

48. Midsagittal section of the embryonic keel and of the closing gastropore, showing the fully developed embryonic and extraembryonic periblast (e. pb. and ex. pb.). The periblastic plug (pb. p.) occupies the remnant of the gastropore and is pressed inside the blastodisc by its closure.

49. Section through the liver of three-day larva. The last vestiges of the oil globule (o. g.) and the periblast (pb.) have been incorporated into the liver where it and several periblastic nuclei (n.) are still recognizable.

PLATE VI

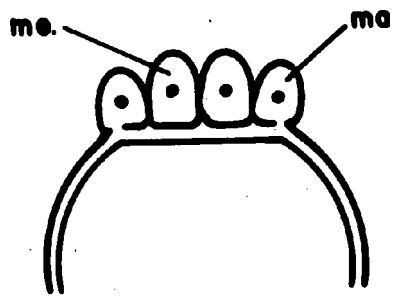


Fig. 42

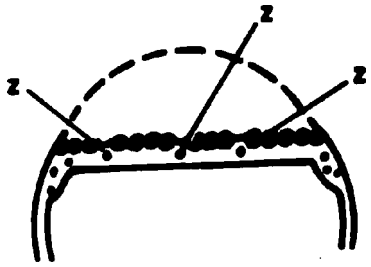


Fig. 46

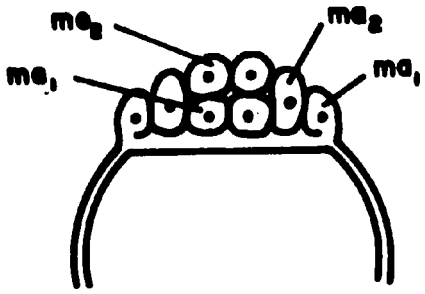


Fig. 43

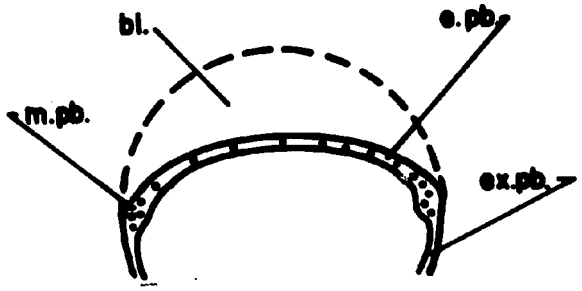


Fig. 47

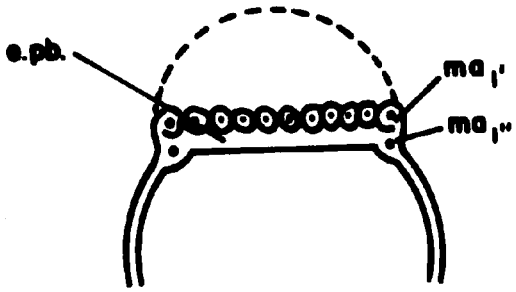


Fig. 44

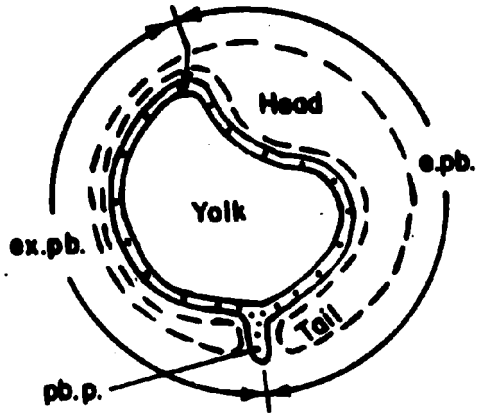


Fig. 48

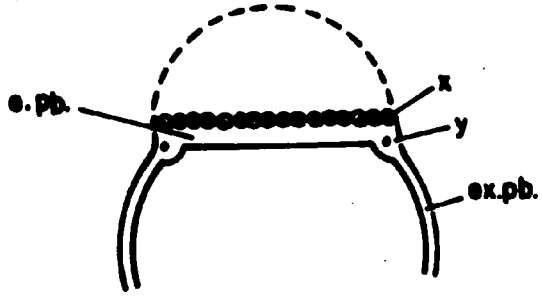


Fig. 45

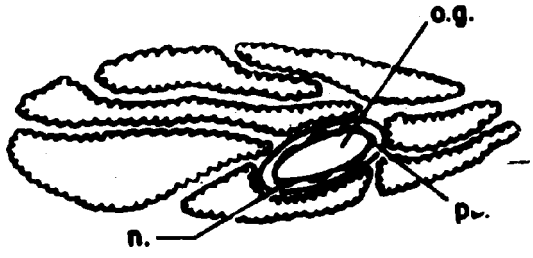


Fig. 49

PLATE VII

FATE MAPS FOR SALMO GAIRDNERI

(From Pasteels, 1940)

Figure

50. Stage 3.8, polar view.

51. Stage 4.0, polar view.

52. Stage 4.5, polar view.

53. Stage 4.7, polar view.

54. Stage 4.9, dorsal view of embryo.

55. Somite stage, dorsal view of embryo.

Dotted area:	lateral mesoblast
White area:	epiblast
Vertical lines:	neurectoblast
Horizontal lines:	notochord
Oblique lines:	somites
Short vertical lines (Fig. 50 only):	entoblast
Solid arrows:	direction of morphogenetic movements in superficial layers
Broken arrows:	direction of morphogenetic movements in deep layers

The contours of the embryo are represented in bold lines and those of presumptive areas are in finer lines. In Figures 50-53, the outer circle represents the rim of the blastodisc and, therefore, the line where involution takes place, i.e. the position of the gastropore. All figures are oriented alike: the lower portion of the outer circle is to the rear of the embryo and represents dorsal lip of the gastropore.

PLATE VII

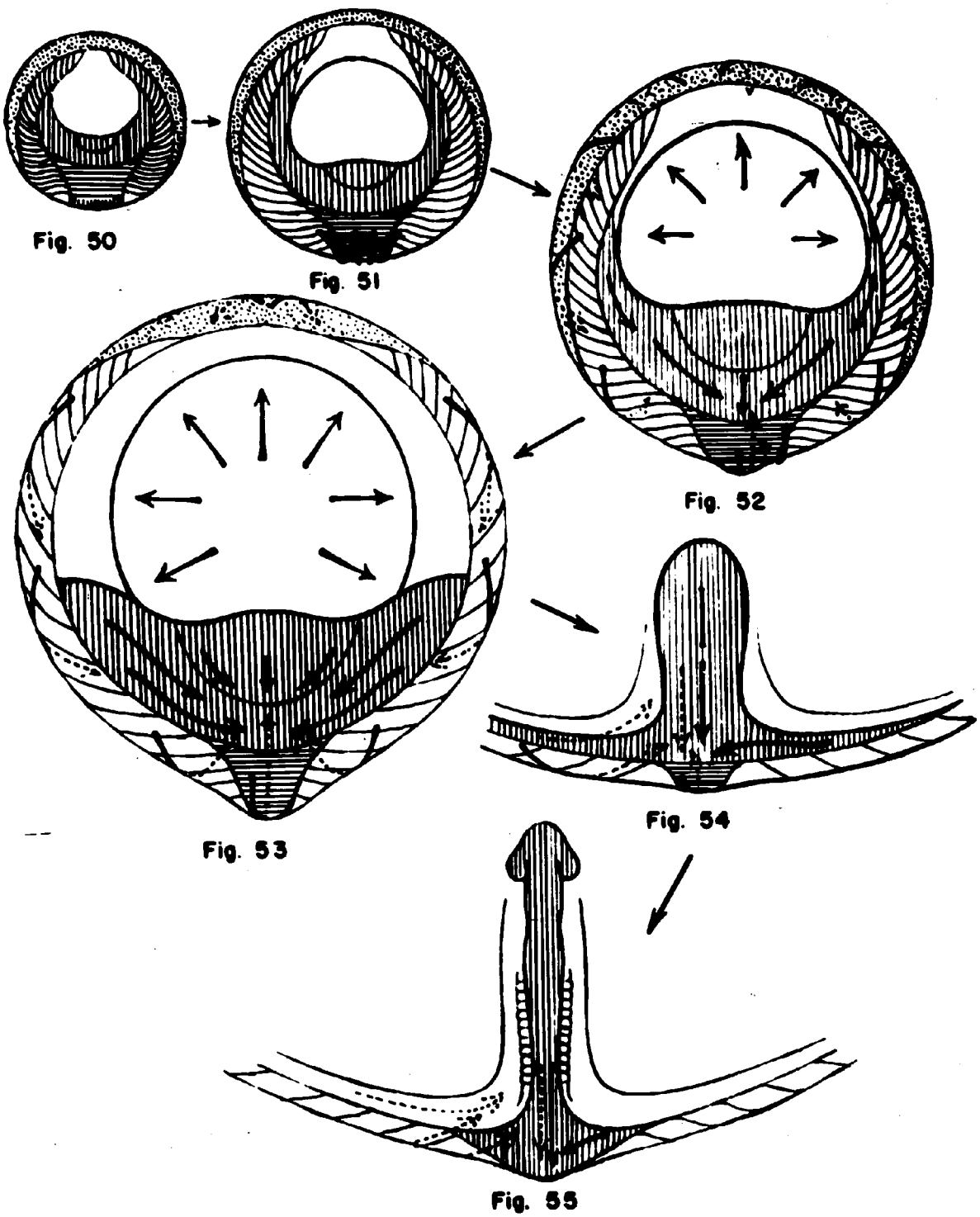


Fig. 50

Fig. 51

Fig. 52

Fig. 53

Fig. 54

Fig. 55

PLATE VIII

PHOTOMICROGRAPHS OF HISTOLOGICAL SECTIONS

Figure

56. Section of stage 2.8, Percina caprodes, x 60. The blastodisc is hemispherical and there is no blastocoele.

57. Section of stage 3.2, Percina caprodes, x 60. The blastodisc is biconvex.

58. Section of stage 3.4, Percina caprodes, x 60. The blastodisc is plano-convex.

59. Sagittal section of stage 3.5, Percina caprodes, x 60. The blastodisc is slightly concave.

60. Section of stage 3.8, Percina caprodes, x 60. The floor of blastodisc is eccentrically concave.

61. Membrane of Percina caprodes egg at stage 3.0, x 1250. Contrast with Figures 13 and 62.

62. Membrane of Osmerus mordax egg at stage 4.9, x 1250. Contrast with figures 13 and 61.

63. Section of stage 2.7, Brachydanio rerio, x 100. showing on the right a marginal cell now in the periblast.

64. Sagittal section of Osmerus mordax, x 60, showing the periblastic arm extending deep into the yolk. No periblastic arm was found in any other specimen.

65. Sagittal section of stage 4.5, Osmerus mordax, x 60, the dorsal lip is on the left and the ventral lip is on the right.

PLATE VIII



Fig. 56



Fig. 61

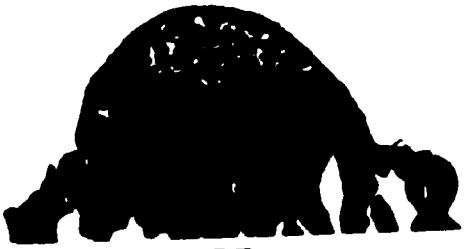


Fig. 57



Fig. 62



Fig. 58



Fig. 63



Fig. 59



Fig. 64



Fig. 60



Fig. 65

PLATE IX

STAGE 4.9 IN PERCINA CAPRODES AND IN OSMERUS MORDAX

Figure

66. Percina caprodes, enlargement of the midventral part of Figure 69, x 400.

67. Percina caprodes, enlargement of the midventral part of Figure 70, x 400.

68. Percina caprodes, enlargement of the midventral part of Figure 71, x 400.

69. Percina caprodes, transverse section through the head, x 100.

70. Percina caprodes, through the trunk, x 100.

71. Percina caprodes, through the tail, x 100.

72. Osmerus mordax, through the head, x 100.

73. Osmerus mordax, through the trunk, x 100.

74. Osmerus mordax, through the tail, x 100.

PLATE IX

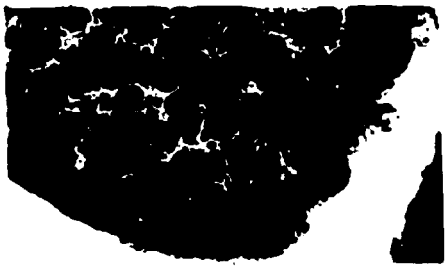


Fig. 66



Fig. 67

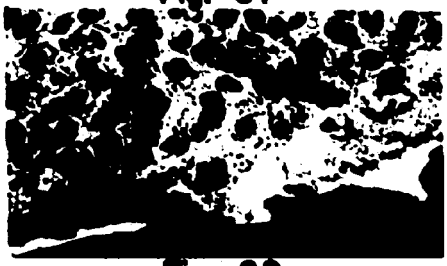


Fig. 68

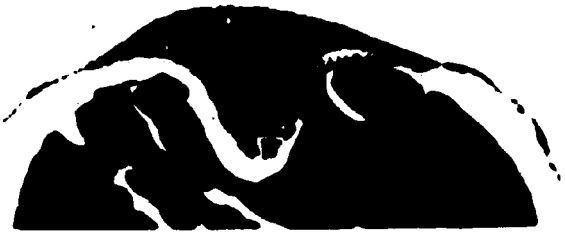


Fig. 69

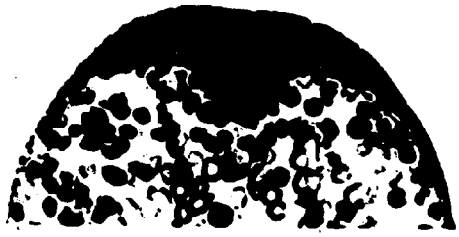


Fig. 72



Fig. 70



Fig. 73



Fig. 71



Fig. 74

PLATE X

MORPHOLOGICAL CHANGES DURING GASTRULATION IN PERCINA CAPRODES

Figures

75-79. Midsagittal sections through the embryonic axis at stages 3.9, 4.0, 4.5, 4.7, and 4.9, respectively.

80-82. Transverse sections through the middle of the blastodisc of stages 3.9, 4.0, and 4.5, respectively.

83. Transverse section of the blastodisc at the tip of the embryonic shield of stage 4.7.

84. Transverse section of the blastodisc at mid-trunk level in stage 4.9.

bl.	blastodisc
c.mes.	notochord
c.pb.	embryonic periblast
d.l.	dorsal lip
em.sh.	embryonic shield
e.pb.	embryonic periblast
epi.	epiblast
ex.a.	extraembryonic area
ex.c.	extraembryonic cavity
g.r.	germ ring
i.cm.	involuting chordamesoblast
i.l.m.	involuting lateral mesoblast
l.l.	lateral lip
not.	notochord
pd.	periderm
v.l.	ventral lip

PLATE X

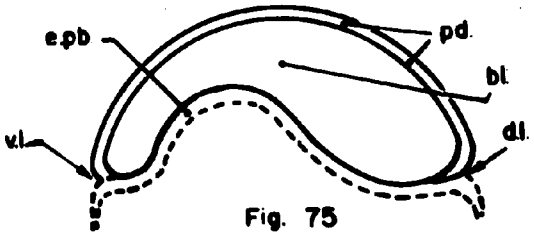


Fig. 75

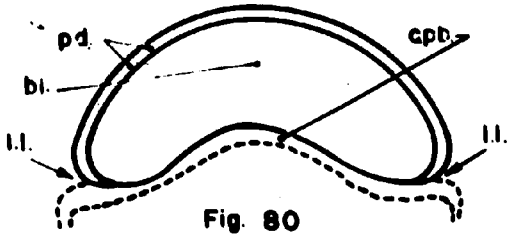


Fig. 80

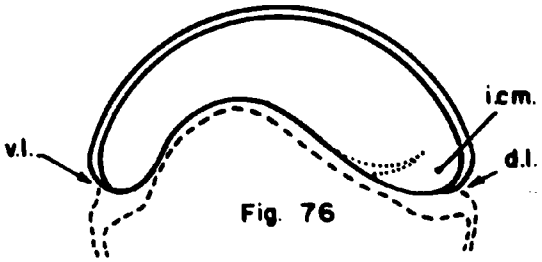


Fig. 76

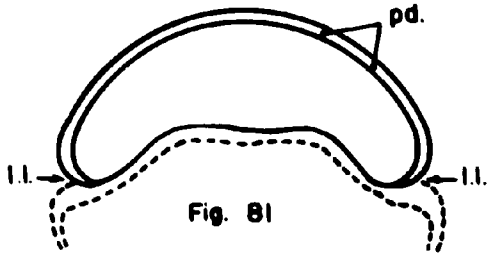


Fig. 81

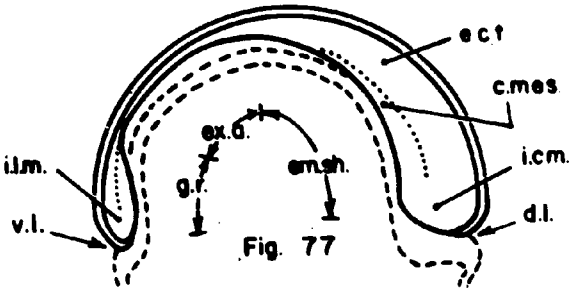


Fig. 77

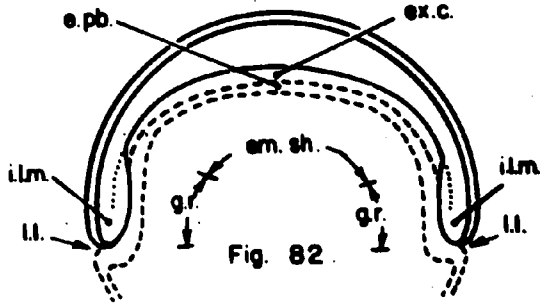


Fig. 82

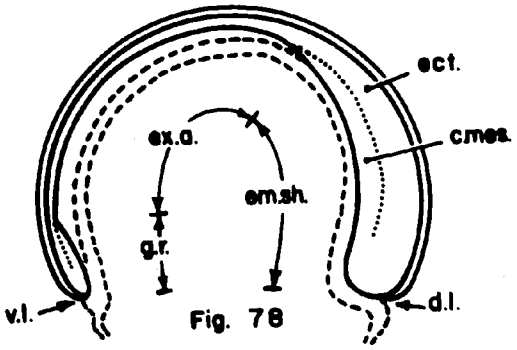


Fig. 78

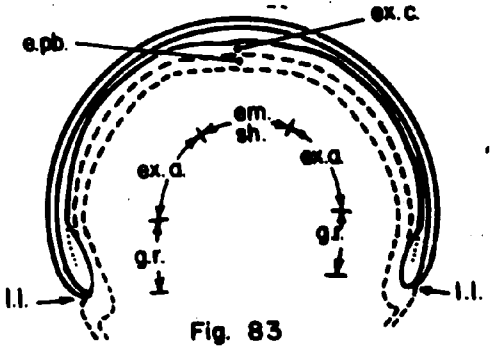


Fig. 83

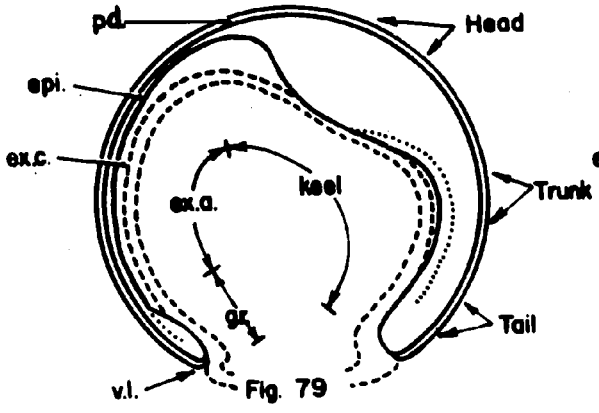


Fig. 79

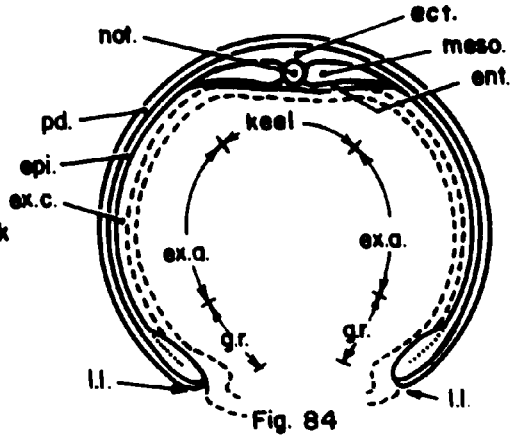


Fig. 84