DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO
IN THE MARYLAND BROOK LAMPREY
(*LAMPETRA AEPYPTERA*, sup.)

by

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of the University of Maryland in partial fulfillment of
the requirements for the degree of Doctor of Philosophy

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CHAPTER I

INTRODUCTION

1. Occurrence of the Maryland Brook Lamprey

The Maryland Brook Lamprey, herein provisionally called *Lampetra aepyptera*, has been found in the last 3 years (1950-1952) by me in Crow and Bear Branches, about one and one-half miles southeast of Laurel, Maryland. These 2 streams are tributaries of the Patuxent River (Fig. 1). Bear Branch appears to represent the Laurel Run of the U. S. Museum records of the early 1900's, and now flows into the artifically created Laurel Lake. The outlet of Laurel Lake is a spillway adjacent to a dam and about 50 yards west of U.S. Highway #1. The overflow from the lake forms a brook which passes under the foregoing highway and its waters are joined by Crow Branch at a crossing of the Baltimore and Ohio Railroad, about one-fourth mile east of the highway.

Over a mile of each of these branches has been explored systematically in the wooded region west of Highway #1. The water movement is slow in the deeper parts of these streams, but is faster over riffles. The bottoms are chiefly fine gravel in quiet sections, with coarse gravel at the bottom of fast-running water. The bottoms of pools (about 3 to 4 feet deep) consist of fine sand, and backwater areas have fine sand overlaying decomposing organic material. Both brooks appear to be free from pollution in the area explored; algae, protozoa and insect larvae are found in abundance.

The holotype of L. aepyptera is stated to have come from Portsmouth, Ohio several years before 1860; it was placed by Mr. C. C. Abbott in that year in the cabinet of the Academy of Natural Sciences in Philadelphia (voce Mr. Henry W. Fowler of the Academy, 1952). What appears to be the same lamprey has been collected from Bear Branch (= Laurel Run) or collateral tributaries of the Patuxent River since April 9, 1888 (U. S. Museum #39,570, G. Marshall). Mr. G. Marshall, together with Ernest B. and H. Marshall, systematically collected the Maryland Brook Lamprey in this locality for several decades (U. S. Museum #49,136, April, 1899; #143,898, April 19, 1902; #143,897, April 23, 1902; #73,882, April 21, 1912 (Crow Branch); #93,870 in June, 1932 and #106,583 on April 18, 1937). Other specimens in the U. S. Museum were from the Paint Branch (likewise a tributary of the Patuxent River) north of College Park, Maryland (#143,899, April 13, 1930, Miss Cochran); Austin's Run (a tributary of the Rappahannock River), at U. S. Highway #1, south of Fredericksburg, Virginia (#100,276, G. S. Myers and E. W. Bailey, April 28, 1935); Suitland Bog, near Suitland, Maryland (#102,362, Dr. Paul Bartsch, about May, 1936); and in runs in Anne Arundel county, near Annapolis, Maryland (#103,760, William H. Bayliff, in the spring of 1937).

Raney (1941) found L. aepyptera in the Neuse River system in North Carolina, and specifically in a tributary of the

Little River on April 6, 1940. Subsequently Bayliff (1942, and personally to the writer, 1951) studied specimens of this lamprey taken from a small tributary of the North River, parallel to Rutland Road at its junction with U.S. Highway #50, near Annapolis, Md. (See also Vladykov, 1950, p. 77). Mr. Romeo Mansueti of Baltimore, Md., reports L. aepyptera in Chambers Lake at Federalsburg, Md., October 26, 1950 (correspondence).

Subsequently the writer and others have found over 400 specimens of adult and larval forms of the Maryland Brook Lamprey in various stages of development in Crow and Bear Branches. To this writer's knowledge, no other species of lamprey has been reported in these brooks.

2. Adult Morphology

. General Characteristics

The adult Maryland Brook Lamprey is a slender, eel-like, somewhat fountain-pen-shaped animal which swims agilely in clear fresh-water brooks (Figs. 2 and 3). It is dark grey-brown to buff dorsally, and white to very light salmon-buff ventrally and over the spiracles. It may grow to 130 mm. (about 5 inches) in length and 10 mm. (about 3/16 inch) in body height. Without jaws, it has a roughly circular sucking mouth, by which characteristically it attaches to the substrate.

There is no complete description published of the Maryland Brook Lamprey. It is practicable, therefore, to give some of the more important morphological criteria by which this lamprey may be identified. Specifically, it is a monorhine vertebrate of elongate, subterete body form, without pectoral and pelvic girdles and fins, but with 2 well-separated posteriorly disposed dorsal fins (the anterior imbricate over the origin of the posterior on the right side). The posterior fin is the higher. The animal has an oval to nearly circular, toothed buccal cavity; an unpaired, dorsally placed nasal aperture; typically paired eyes; and 7 exposed branchial clefts sublaterally placed behind the eyes. There is a distinctive, diamond-shaped caudal fin. The male has the long gonoducal papilla characteristic of the genus, and it has a larger buccal cone than the female, with coarser fimbriae and teeth.

B. Dimensions

No extensive statistical coverage of the total length of the adult or metamorphosing lamprey has been made. Measurements made upon relaxed animals in chloretone water show:

TABLE

Kinds of Lampreys	Number of Animals	Lengths in Millimeters		
		Average	Maximum	Minimum
ADULTS				
Total	22	103.7		
Male	12	103.8	120.0	93.0
Female	10	103.6	119.0	90.0
METAMORPHOSING ANIMALS				
Total	28	107.4		
Male	8	105.1	125.5	90.0
Female	20	108.6	130.0	93.0

TABLE I (Continued)

AMOUNT OF SHORTENING, AVERAGE LENGTHS: (Base = Average, Metamorphosing Animals)	Total	3.4
	Male	1.2
	Female	4.6

It is seen that the metamorphosing animals have undergone an average shortening of 3.4% upon attainment of the adult state. The change from larval form begins internally about October 15 and externally about February 1 of the following year, at the 39th parallel.

Variation in lengths of individual animals is thus very great. One maturing female caught during this period was, as indicated, 130 mm. in overall length. At the same time there was maintained in the laboratory another female which had been caught as a larva October 13, and which at its death on the following March 17 was far advanced in metamorphosis. Because of its over-winter environment in the aquarium, this relatively unfed animal was only 82 mm. long on March 1. It appears, therefore, that the longitudinal growth of the late larva is significantly dependant upon ecological conditions, particularly upon the availability of food.

At this point it is convenient to mention that 70% ethyl alcohol used as a preservative caused considerable shrinkage in the lengths of these animals, chiefly because of myotomal contraction. Sixteen animals so fixed contracted from an average length when living of 109.3 mm. to 99.3 mm. in alcohol (9.2% of the original length). In contrast, the killing and preservation of 7 animals in half strength formalin (18.75%)

caused a very small average decrease of 0.2 mm. (from 104.2 mm. to 104.0 mm.).

C. Myotome Count

One of the criteria considered to separate this species from others is the average number of muscle segments which may be counted from the seventh (most posterior) spiracle to the beginning of the anus. This count has been given by Fowler (1907) to be about 53; and counts for the late larva averaged 54 (Vladykov, 1950). 40 adults and 15 late larvae collected by me show:

TABLE II

# Myotomes	# Adults	# Late Larvae (Over 100 mm. long)
53	1	-
54	-	-
55	7	3
56	8	4
57	19	7
58	2	-
59	1	-
60	2	1

The average myotome count appears to be 57±3. The myotome count of the holotype, as personally made by me, was 57. In making such counts it has been borne in mind that ventrally the muscle segments are reflexed anterad, and those immediately in front of the anus are strongly compressed. Consequently the more lateral, wider part of the last 3 or 4 segments may seem to be located posterior to the beginning of the vent.

D. Coloration

The coloration of the adult Maryland Brook Lamprey is derived from, at least:

i. Stellate melanophores, capable of changing considerably in size. These are disposed in a single layer just below the somewhat transparent epidermis, and are greatest in number in the dorsal midline. They may extend in patches on the ventroposterior part of the animal, giving it a piebald appearance. Anteroventrally, there are few melanophores. In many animals the melanophores are surrounded by other cells whose cytoplasm is filled with a finely distributed, deep brown pigment, which gives the area a very even, deep raw-umber color.

ii. Very small cellular inclusions which reflect a coppery sheen. These are extremely minute and more or less evenly distributed in the cells just under the epidermis.

iii. Ventrally, the glistening white of the belly is caused by numerous microscopic, scale-like cell inclusions, which appear to be similar to those occurring, for example, in the epidermis of the Clupeiformes. The whitening of the venter is an indication of maturity; this is reinforced when the skin above the spiracles likewise becomes quite pale.

iv. The bluish tone arising from the breaking up of light in the imperfectly transparent epidermis, and which modifies the brown coloration. This contributes to the grayish color of many animals, particularly when the melanophores are contracted.

E. Dentition

The dentition of the Maryland Brook Lamprey appears to be characteristic of that described for the genus Lampetra (Regan, 1911; Creaser and Hubbs, 1922; Berg, 1931). See Fig. 4.

Located just within the oral fimbriae there is a single circular series of small, blunt teeth (arciform in cross-section), averaging about 65. These frame the oral cavity and may be termed the border teeth; ventromesially they become smaller.

Immediately above the entrance to the throat is the supra-oral lamina, which in the recently metamorphosed adult consists of 2 stout, obtusely pointed cusps whose points are directed centrally and downward. These are connected by a bridge which may be partly covered by the buccal epithelium. These cusps are somewhat variable in shape; in one animal one cusp had been developed centrad so that instead of terminating in a blunt point, the anterior edge was chisel-like. In old animals the cusps are worn and are sometimes fractured. They are usually light buff in newly-metamorphosed animals, but darker tones have been observed in older animals.

The infra-oral lamina, which is located on the posterior internal border of the buccal funnel (opposite the supra-oral lamina) is broader than its upper counterpart. It is band-like, the long axis of the lamina lying in the horizontal plane. The outer edge of the lamina is quite variable in design, but it is usually divided into 5 or 7 very blunt "humps", which may be designated for convenience as reduced cusps. The 2 distad cusps on each side appear to be fused when only 5 may be counted; those outside cusps are then usually very large. Infrequently the infra-oral lamina is quite band-like.

Laterally disposed at each side of the disk are 3 single or double cusps, arrayed in parallel with the lateral curvature of the buccal border. They lie near the throat, and are larger than those in the rest of the buccal cone, excluding the laminae. Each tooth of these sets has at least 1 cusp; and animals are found wherein the anterior- and posteriormost teeth were bluntly bicuspid. The number of cusps appears to be variable.

On the anterior face of the buccal cone, between the supra-oral lamina and the anterodorsal border teeth, there are 3 rows of teeth, all unicuspid, and which exhibit a fairly constant morphological arrangement. From outside towards the pharynx, the number of teeth in each row is 2, 5 and 4.

There appear to be no teeth on the posterior face of the buccal cone between the infra-oral lamina and the border teeth. This observation is based on microscopic examination (970 x) of complete serial longitudinal and cross-sections of this part of the adult animal, cut at 8 u.

F. Other Criteria

In the first published description of *L. aepyptera* by Abbott (1860), no reference is made to certain morphological characteristics found in the Maryland Brook Lamprey.

Also, there is some variation in the structure of the Maryland form from that published for the alleged holotype. It is beyond the scope of this paper to enter into a critical taxonomic discussion, but certain of these differences should concern the interested taxonomist and for that reason they are mentioned here.

First of all, the holotype is somewhat larger than the average length of this Maryland Brook Lamprey. It was originally reported in 1860 to be 5½ inches long (140 mm.), which seems to have been its original length. Fowler (1907) found this animal to be 4-7/8 inches long (123.8 mm.); therefore it had undergone contraction of more than 16 mm. in the nearly 50 years which had elapsed since Abbott's observations. Mr. Fowler did not collect new material from this locality at the time. Only 6 Maryland lampreys (12% of those measured) have exceeded this later value. Compared with the original reported length (140 mm.) of the holotype, the average length of the Maryland form is shorter by about 36 mm., and none has been found of 140 mm. length. Whether this is (a) a genetic difference, (b) a reasonable ecological variation, or (c) an actual species or subspecies difference remains to be determined.

In the redescription of the holotype by Fowler (1907) he states that there are about 53 myotomes between the posteriormost spiracle and the anus. This seems to be a matter of definition, as I found 57, as hereinbefore noted. Therefore, the myotome count in both the holotype and in the subject lamprey is about the same.

In Abbott's description of the fins of *L. aepyptera* it is stated that

> The first dorsal fin arises somewhat posteriorly to the centre of the entire length of the body, and is pyramidal in figure. The second dorsal fin, joining with the caudal, is smaller than the first dorsal and more acutely pyramidal in its figure. The caudal, whose origin is situated opposite the vent, is higher than either dorsal fin, and decreases rapidly towards the tail.

Part of this description of the holotype does not appear to be correct, as the specimen itself will show. My observation (1952) was that the caudal fin is not higher than either dorsal fin; it is lower than both. From its present condition it is not readily possible to determine the relative height of the posterior dorsal fin of this ancient specimen; but a very serious doubt may be cast upon the accuracy of Abbott's statement that the anterior dorsal fin is the higher one of the two. The enthusiastic calipers of many researchers have reduced this old cyclostome to a sad state of dilapidation, and the dorsal fins are badly frayed. In any case, paratypes as accepted in the U. S. Museum show that the second (posterior) dorsal fin is higher than the first (anterior). The same holds true for all adult specimens of the Maryland Brook Lamprey that I have caught. It is certain, then, that this part of Abbott's description cannot be used safely to define the fins of *L. aepyptera*.

The prolonged snout is also described as one of the peculiar characteristics of *L. aepyptera*. Snout length is measured here by the distance from the middle anterodorsal edge of the snout to the beginning of the first spiracle. In the holotype this is 13 mm. (*voce* Henry W. Fowler, March 27, 1951). In the Maryland Brook Lamprey this measurement for 10 completely mature animals averaged 11.2 mm., or 13.8% shorter; in 2 animals, however, snout length was 13.0 mm. or more.

L. aepyptera has been effectively distinguished from *Entosphenus lamottenii* (*Entosphenus appendix*) by Creaser (1939). In any case, plate I in Creaser and Hubbs' *A Revision* of the *Holarctic Lampreys*, identified as *Lampetra* (*Okkelbergia*) *lamottenii* (Creaser and Hubbs, 1937, facing p.14) does not portray correctly the dentition or facies of this Maryland Brook Lamprey.

Therefore, with all of the foregoing in mind, I think that only a provisional classification of the Maryland Brook Lamprey as *L. aepyptera* seems practicable at this time.

3. *Life History*

The adult Maryland Brook Lamprey spawns in Crow and Bear Branches from about April 10 to May 10, possibly earlier. Adults can be found for several days afterward, but they become progressively moribund, and soon die. They are similar to other non-parasitic brook lampreys in that the intestinal tract degenerates at maturity to a threadlike, useless strand.

In the experience of this observer, the females caught (usually between 1 and 5 in the afternoon) seem to complete their metamorphosis before the males. Likewise there are caught about 3 females to every 2 males; in the latter part of March and the first week in April the ratio is even greater: 3 to 1. Only in May are more males found.

The water of the brook may be as cold as 11° C. when the adults start to come forth from the brook bottom, but spawning actually takes place when the temperature has risen to approximately 16° C. The lamprey selects a very shallow place where the water runs more slowly, and in this case has been found in naturally occurring ripple-like depressions in fine, graded gravel of the brook bottom, a little distance above riffles. This lamprey has been seen to transport stones, but its attempts at nest building are relatively uncoordinated. I have examined 2 nests, both hollowed out adjacent to a somewhat large stone (the latter perhaps 2 inches in diameter). No array of pebbles suggestive of artificial arrangement has been found; and spawning activities in laboratory aquaria under fairly close observation exhibit only sporadic attempts at picking up sand.

The nesting lamprey attaches itself by its mouth to a large pebble on the site selected, and with rapid vibratory motions of the posterior half of the body, "fans" the fine gravel from underneath its body, creating an oval, bowl-shaped depression. The pairing lampreys both join in this activity. Such nests are not very deep, perhaps to a depth of 3 inches below the plane of the local brook bottom; they are about 7 or more inches in the longer diameter.

This activity merges into the spawning behavior. During nesting activity the labia of the anus of the female become bloodshot; and the fins of both lampreys may become flecked with petechiae. In preliminaries to the sexual act,there has been observed a sort of sexual play as exhibited by the animals maintained in laboratory aquaria. This consists of a characteristic, slowly undulate swimming; the ventral side of the trunk rubs against the gravel, while the two animals interweave in and out. Eventually the male attaches dorsally to the head of the female (or to the stone or aquarium wall to which the female has already attached). In a sudden motion he curves the posterior part of his body sharply in a complete circle about the female, and brings the penile papilla into apposition with the reproductive aperture of the female. Anteriorly his body is arched tautly over the female, and that part of his body which encircles the female vent contracts convulsively at least once, perhaps a number of times. Concomitant with this there is a rapid and violent vibration of the body, particularly in the caudal region of both animals. The act is periodic and lasts for about 3 to 5 seconds at a time, with resting periods of variable extent in between. During this act the female extrudes one or more individual batches of 2 to 4 eggs, rarely more and usually 3. At the time the female lays the eggs the male fertilizes them. The spawning movements are so fast that it is not possible to determine whether or not a form of intromission is achieved. Spawning in the laboratory usually occurs in subdued light or at night. A gravid female contains over 1,100 eggs (1,164 in one case by actual count), and practically all of them are layed.

Upon fertilization the jelly about each egg expands. The egg falls to the brook bottom where it is soon covered by fine particles of gravel which adhere strongly to the extremely viscid outer jelly. The flurry of gravel caused by the spawning motions of the parents, as well as the movement of gravel caused by the changing flux of the stream, may cover the eggs and protect them.

In my aquaria it took about 265 hours for the 4 mm. cream-colored larvae to emerge from the eggs (temperatures ranged from about 18° to 22° C.). In the brook they are either then caught in the changing water currents or swim to fine silt beds in very quiet (but not stagnant) water, and which are dark from the presence of thoroughly decomposed leaves and other organic matter. In this locale the developing ammocoete appears to stay throughout its first year. By October 15 of this year it has achieved an average length of 34 mm., and no animal of this generation was dug up at the time that measured less than 28 mm. Animals up to a length of 43 mm. (at least) appear to be first year larvae. This is based on a group of fifteen animals taken on that date.

The progressive longitudinal growth of the larvae is illustrated in Tables III and IV. The indicated life span is three years. An extensive sampling made on February 2 uncovered no larva shorter than 33 mm. length, which is almost the average for the October 1 - 28 sample. It is safe to conclude, therefore, that these smallest of the February larvae constitute first yearlings. In the say 168 days elapsed since fertilization, the growth of 34 mm. in length represents an average of 1 mm. every five days (it is recognized that this

TABLE III

Longitudinal Growth in Millimeters
Larvae and Adults of the Maryland Brook Lamprey

Frequency Distribution of Animals
By 10-Millimeter Increments and 14-Day Periods
Beginning April 2 and Ending the Next April 1*

Consecutive 2 - Week Periods	1 to 10 mm.	11 to 20 mm.	21 to 30 mm.	31 to 40 mm.	41 to 50 mm.	51 to 60 mm.	61 to 70 mm.	71 to 80 mm.	81 to 90 mm.	91 to 100 mm.	101 to 110 mm.	111 to 120 mm.	121 to 130 mm.
Apr. 2 - 15	#8									a 1	a 1	a 5	
Apr. 16 - 29									a 1	a 4	a 4		
Apr. 30-May 13	#4								a 1	a 2	a 3		
May 14 - 27							3	3		1	1		
May 28-June 10	n	n	n	n	n	n	n	n	n	n	n	n	n
June 11 - 24	n	n	n	n	n	n	n	n	n	n	n	n	n
June 25-July 8		7		4	6	8	1		3	5	1		1
July 9 - 22		9	4	4	7			1					
July 23 -Aug.5	n	n	n	n	n	n	n	n	n	n	n	n	n
Aug. 6 - 19		5	3	1	3	3	3	4		3	1		
Aug. 20-Sep. 2		4	5	2	2	4	2	2		1			
Sep. 3 - 16				1	1								
Sep. 17 - 30		3	4	2	3	7	3	4	2	6	3	1	1
Oct. 1 - 14										3	1	2	3
Oct. 15 - 28			2	12	1				2		2		1
Oct. 29-Nov.11	n	n	n	n	n	n	n	n	n	n	n	n	n
Nov. 12 - 25												1	
Nov. 26-Dec. 9	n	n	n	n	n	n	n	n	n	n	n	n	n
Dec. 10 - 23	n	n	n	n	n	n	n	n	n	n	n	n	n
Dec. 24-Jan. 6	n	n	n	n	n	n	n	n	n	n	n	n	n
Jan. 7 - 20	n	n	n	n	n	n	n	n	n	n	n	n	n
Jan. 21-Feb. 3				6	12	3	5	3	5	13, 1m	13, 2m	2, 1m	
Feb. 4 - 17	n	n	n	n	n	n	n	n	n	n	n	n	n
Feb. 18-Mar. 3										2, 1m	6, 8m	1, 2m	2m
Mar. 4 - 17						1					5		
Mar. 18-Apr. 1					7	3	1	2	5, 1m	3, 2m	1, 7m	1, 1m	

Key

a = adult
m = metamorphosing animal
n = no collection of animals was made

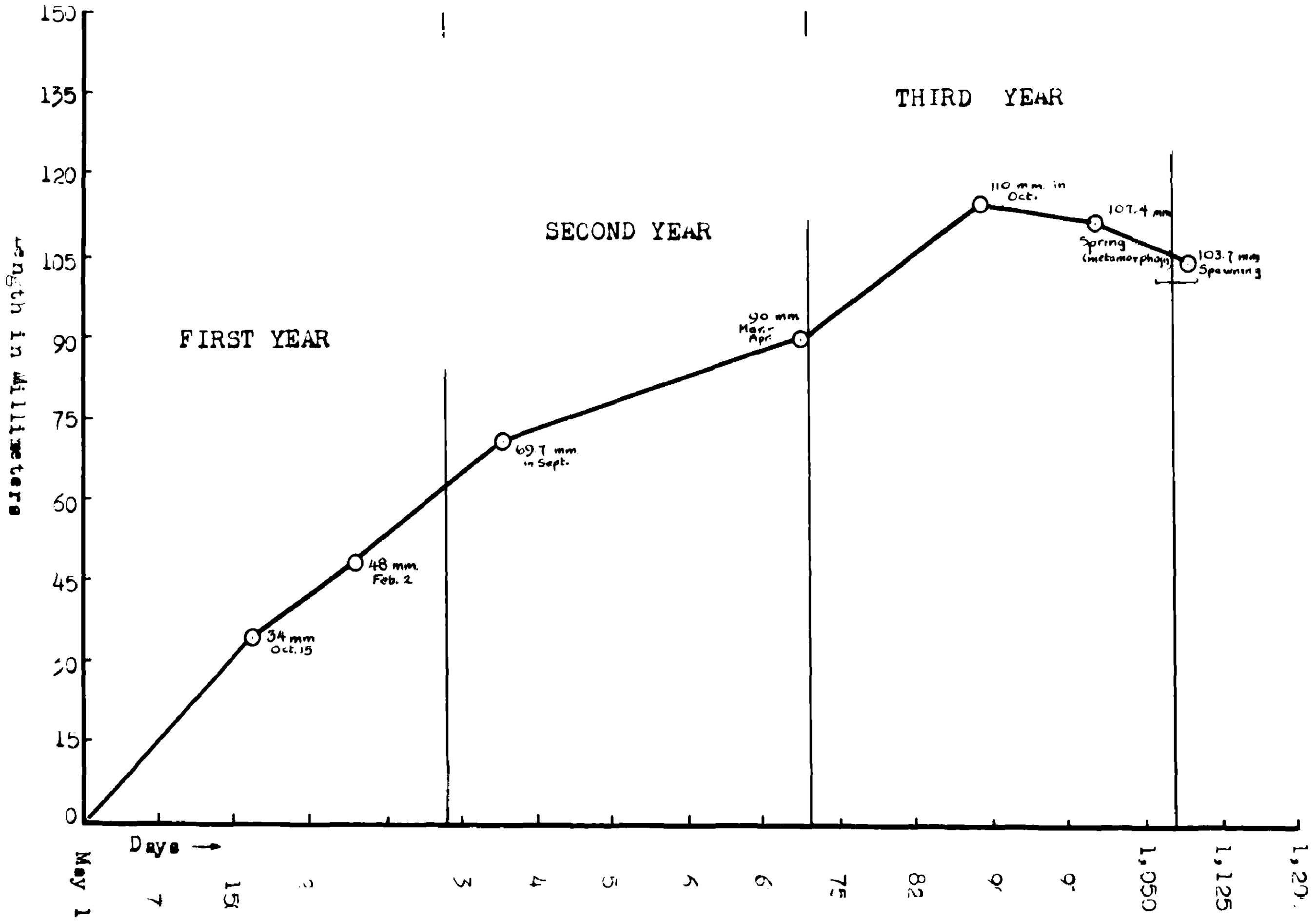
Note: Numbers not annotated represent larvae caught. Vacant rectangles mean that trips were made during this time, but that no animals of the length in question were caught.

*Not all animals caught were measured.
#Hatched in laboratory aquaria.

TABLE IV

INDICATED GROWTH OF THE MARYLAND BROOK LAMPREY

Average Longitudinal Length



is probably not a straight line trend). If this indicated growth rate were continued throughout the winter months, on February 2 (110 days later) the average indicated length of these first yearlings would be 62 mm.

The 67 lampreys collected on February 2 fall into 2 clusters, separated by a gap roughly in the 67 to 77 mm. interval. In the first cluster the animals range in length from 33 to 66 mm. (there is one larva of 71 mm. length of doubtful category, which has been excluded from computation). The wider dispersion of lengths may be expected as indicated by the development of the "spread" found in October. The weighted average of this distribution is 48 mm., which shows that there is a definite slowing down of the rate of longitudinal growth in colder weather.

This retardation in growth rate seems to be maintained until well along in the year. In the middle of August, however, the average length of 12 larvae was 64.4 mm. This cluster has become, with the advent of the new generation in April and May, the second year group. By the last of September, 18 animals considered to be referable to this group had increased in mean length to 69.7 mm. It is this group, with a range in length of about 57 to 84 mm., which in turn forms a part of the second cluster of February 2.

This second cluster is believed to consist of 2 converging groups of animals: (a) those that are about to metamorphose (to spawn in April and May), and (b) those which will form the late larvae of the third year. This distinction is well supported: in May, June and July, long after the adults of April and May have spawned and died, these larvae of practically adult body length are to be dug out of the fine gravel bottom. Of the 40 lampreys in the February 2 group, 4 were obviously metamorphosing. Because of limitations of time it has not been possible to autopsy each animal to determine the condition of its reproductive tract; however, sufficient examinations have been made to establish very definitely that there are two stages of development existing here: one where the reproductive strand is a fine, undeveloped cord of cells and the other where the gonads have undergone most of the maturation process. The mean length of the apparently non-metamorphosing lampreys of this date is 97.5 mm., although it is to be borne in mind that this group without doubt includes animals which will transform shortly, as explained further below. Based on the preceding year's trends, the computed value would be about 87 mm. for non-transforming larvae.

The changes in length of metamorphosing near-adults and lengths of adults are discussed on page 6. For non-metamorphosing larvae, the weighted average length of 12 larvae on March 20 (the close of their second year) was 90 mm., which is almost precisely the indicated trend. Since transforming animals become adults in April and May, all remaining larvae of approximately these lengths represent third-year ammocoetes, which become adults in the fourth April or May of their existence, at the end of their third year. In early

October these third yearlings are, on the average, about 110 mm. long, practically the length of metamorphosing animals in their final spring months.

During their development the ammocoetes are found in different environments. The fine, mudlike silt of early larval life is exchanged in the second year for fine, sandy drifts, particularly under the lee of banks where the conformation of the brook protects the sand from the thrust of water flow. In the third year they are found in somewhat coarser sand, but they are still much more frequently dug up near the banks of the stream. In late winter and early spring these second- and third-year larvae may be found in great number between layers of fallen leaves of the previous fall, particularly where the stream has piled sand over them.

The metamorphosing larva of February and March often buries its body up to its posteriormost spiracle in the brook bottom,but leaves its head clear in the water. Such animals orient themselves facing stream flow and may be found anywhere in the fine gravel stratum of the brook bottom. Here they stay presumably, until the urge to mate sends them to their nesting grounds.

The Maryland Brook Lamprey evidently does not have to leave its brook during its lifetime. In the case of the present example, all stages have been found in a mile-long stretch of the brook. It is true, however, that as the larvae grow older they inhabit parts of the Branch progressively nearer the lake, and they may occur in it.

CHAPTER II

MATERIALS AND METHODS

. Living Material

A. Procurement

Equipment required for the procurement of living material includes a standard-sized shovel, a fine-meshed, large fish-net, a small aquarium fish-net, 4 gallon collection jars, and hip boots. Also indispensable are a Centigrade thermometer for measuring water temperature, a camera, and a note book for records.

Larval forms of this lamprey are almost never found on the surface of the brook bottom except when metamorphosing. Usually they are found within 12 inches from the bottom surface, and careful spading and combing of silt, sand or fine gravel of uniform composition, particularly at the edge of the brook, is frequently productive. Coarse or pebbly, hard gravel bottoms are sterile. The material spaded is deposited with a spreading motion on the bank; often the larvae will squirm to the light. Since the larval skin is slimy, collection is aided by scooping the animals into a small, fine-meshed aquarium net.

The larvae seem to be somewhat gregarious, and a favorable location often discloses a community of animals of approximately the same size.

Nets, of course, are indispensable for capture of metamorphosing and adult animals.

B. Maintenance

Larval forms of the Maryland Brook Lamprey have been maintained successfully for periods of a year or more in well-aerated aquaria kept in a cool place. The brook water is replaced occasionally to prevent fouling. Either silt, sand or gravel, as appropriate, is placed on the bottom of the aquarium to the depth of about 2 inches (although less will serve). Detrimental factors encountered in maintenance have been (a) fungal infections with a species of the Saprolegniales, (b) high temperature, and (c) nematode parasitism.* Also, addition of organic matter must be made with caution, because bacteria invariably introduced with it can kill off larvae with almost lightning rapidity.

The adult lamprey has spawned in aquaria in the laboratory. It is preferable to have an aquarium of 9" x 9" x 18" or larger, as the animals are aware of, and do not respond well in a more restricted area. A deep layer (2" or more) of clean, well-washed gravel of fairly uniform texture is placed in the bottom. This gravel is sandy. Over this gravel clean brook water is placed to a depth of about 2 inches. A large stone is, or two are added in opposite corners; an aerator is introduced, and a pair of active adults are put in. The female must be obviously gravid (the eggs can be seen through the ventral wall) and the male "new" and very active. This nesting aquarium is kept in a quiet, moderately lighted, cool place. The spawning pair take a little time to become adjusted upon being placed in the tank and may show a fright reaction, sometimes for an hour or more.

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*Mr. Willard Smith, now at the University of Maryland, has discussed the classification of a nematode found in the Maryland Brook Lamprey with Mrs. M. B. Chitwood. It appears to be a new species in the family Cucullanidae.

During, or just after spawning activity, the eggs are removed by a wide-mouthed medicine dropper to covered specimen dishes which are two-thirds filled with filtered brook water. These are then placed in wide, shallow trays in which tap water is allowed to flow continuously, to maintain a low temperature of about 16° to 22° C. The brook water does not need to be aerated until after hatching. Shortly after hatching, the young larvae are immersed in larger bowls in which fine silt has been placed, together with an aerator.

2. Fixation and Associated Procedures

Two methods have been used to stupefy and subsequently kill lampreys when it has been desired to operate upon the relaxed animal. Another method, incident to a special embedding technique (described under 3. Embedding, Mounting and Staining) freezes and kills them instantly. These are:

i. Placing frozen carbon dioxide in the water in which the animals swim, until the water is thoroughly saturated and the temperature approaches 0°C. (the water should not be allowed to freeze).

ii. Preparation of a saturated aqueous solution of chloretone (1, 1, 1 trichloro-2 methyl propanol-2). This solution is pipetted slowly into the water containing an animal until the latter no longer actively responds to touch.

iii. Cutting sections of the live animal directly with sharp scissors and as swiftly as possible; and then freezing by immediately dipping the sections in liquid air. The latter is kept in cylindrical Dewar flasks adapted for the purpose, and sections are manipulated with long forceps.

The first two methods have proved useful in the performance of laparotomies to dissect out the reproductive tract, or to cut large animals into units of suitable size for fixation with chemical agents. The first method is preferred for cross-sectioning the animal into small blocks for ready fixing, and the second method for dissection and measurements. I have just started to use the third method; its objective is to eliminate fixation artefacts which arise in the other techniques employed.

The integument is a remarkably impervious barrier to many of the more delicate fixing agents. The animal's tissues should be cut up into small pieces, except for the pharyngeal region. Larger units or entire animals should have the coelomic cavity well opened.

For general application, the somewhat slow-acting but inexpensive and stable Liquid of Tellyesniczky (Lee, 9th Ed., 1928, p. 42) is a good fixing agent. Bouin's solutions, including the one with urea have been used also, but with surprisingly poor results. Flemming's weak solution produces excellent results.

3. Embedding, Mounting and Staining

A. Material Treated With Solutions Containing Inorganic Salts and/or Organic Compounds

All fixed material has been thoroughly washed. Specimens are then infiltrated progressively with ethyl alcohol, xylene and paraffin (m.p. 58°-62° C.) according to standard techniques. Ribbons have been cut chiefly at 8 μ or at 10 μ when desirable, and mounted on slides with Haupt's adhesive.

Most material has been routinely stained by using the iron-alum hematoxylin procedure, without counterstain to avoid bad photographic effects. 3 other methods have been used on the maturing testis:

1. Wright's stain. The slide with the paraffin ribbon is sent through a xylene-methyl alcohol series to absolute methyl

alcohol and then stained in the same manner as a blood smear. However, instead of allowing the slide to dry, it is rinsed in water, then very rapidly "swished" in absolute ethyl alcohol, then in $\frac{1}{2}$ xylene - $\frac{1}{2}$ ethyl alcohol. The slide is now transferred to xylene, and covered in the usual way. Chromatin stains blue, cytoplasm pink and some tissues intermediately.

ii. Gentian violet, with subsequent treatment with potassium iodide and iodine (Lee, 9th Ed., 1928, p. 172).

iii. Delafield's hematoxylin without eosin. This stain has proved to be the least useful.

B. Material Frozen in Liquid Air

William E. Maloney and the writer have constructed a drying and embedding apparatus which is a development of that used by Wang and Grossman (1949). The detailed construction of this apparatus will be the subject of a future paper, but essentially the system consists of a series (6) of detachable test-tube-shaped large embedding chambers, with ground glass valve connections which are sealed with a silicone vacuum grease. These are connected in series with an appropriate vacuum gauge, a mercury diffusion pump and a forepump; and the glass connecting tubes are interrupted by stopcocks which isolate each embedding chamber as may be required. There is a stopcock which seals the entire system from the outside.

Within each embedding chamber a burette top of smaller diameter than the base of the chamber (hence easily removable) is placed at the bottom, closed end down. This burette top is half-filled with a low-melting (±40° C.) de-aerated paraffin. The lead-off duct to the forepump is shielded by gauze filled with Ascarite, to dehydrate material. Each embedding chamber is immersed in a varsol-frozen carbon dioxide mixture which initially is maintained at -70° C.

At the time material is frozen in a closely adjacent Dewar flask filled with liquid air, an embedding chamber is detached. The well-frozen specimen is swiftly removed from the liquid air, and dropped on the surface of the paraffin. The embedding chamber is immediately returned to the system, and reimmersed in the varsol mixture. Internally both air and water are extracted from the system and the specimen for about 5 days.

After the first day the temperature of the varsol mixture is allowed to rise to about 0°C. but not above this temperature. On the fifth day the varsol mixture is removed and a cylindrical electric heating jacket with a hollow bore greater than that of the embedding chamber is placed around the latter, just above the level of the paraffin. This is connected with a rheostat which controls the temperature. In the vacuum the paraffin soon melts; and the specimen immediately embeds. A successful embedding is indicated when no bubbles issue from the material.

The heating unit is then withdrawn and the paraffin allowed to solidify. Upon accomplishment of this state, air is readmitted to the system, the embedding chamber is detached, and the burette top removed.

A little gentle heat cautiously applied brings out the embedded material in the paraffin block. Upon proper shaping it is ready for the microtome. Very successful presentations have been obtained.

4. Photography

All microphotographs have been made with a Visicam, using Eastman PX-135 36 mm. fine-grained panchromatic film. Prints were enlarged 2½ times unless otherwise stated, using N-3 Kodabromide contrast paper. Direct photography on 36 mm. film was performed with a Thagee Exa camera provided with a portrait attachment.

5. Other procedures

Ovulation in the metamorphosed female lamprey was accomplished prior to spawning by the method of Rugh (1935). A laparotomy was performed on the narcotized animal, and the exposed abdominal cavity, with the ovary intact, was immersed in Holtfreter's solution to which 0.2% hydrochloric acid and 0.2% pepsin had been added. Ovulation of 1,161 out of 1,164 eggs was accomplished within 20 minutes. Eggs were counted immersed in water in a large petri dish, placed over a sheet of quadrille paper to facilitate enumeration.

Microscopic measurements were made with an ocular micrometer which had standardized the powers of the objectives by the use of the usual calibrated slide.

CHAPTER III

DEVELOPMENT OF THE GAMETES

1. First Year Larvae

A. Historical

Lönnberg and others (1924) have reviewed the earlier work, which is summarized here. According to Hatta (1892) in *Petromyzon* the original stem cells from which the reproductive tissues arise are coelomic outgrowths, and hence of mesodermal origin. Goette (1905) agrees with Hatta. Wheeler (1899) was of the contrary opinion that the primordial germ cells descended from endodermal cells. Lönnberg (*op. cit.*) is inclined to agree with Hatta and Goette, on the basis that the work of the latter was more thorough, and likewise that they had "more favorable material." Goette observed the anlagen of the gonads as a cellular strand on each side of the notochord. Sometime later in development both strands are shifted medially, and then are adjacent to the median side of the mesonephroi. Still later, both anlagen fuse to an unpaired central organ, as W. Müller observed in larvae 38 mm. long. That the unpaired structure of the gonads is a secondary development in growth and not a primary one has been stressed by other early writers, such as Duméril (1812), Home (1815), Bory de Saint Vincent (1822), and Rathké (1825, 1827).

In his study of the early history of the germ cells in

Entosphenus lamottenii (= E. wilderi, E. appendix et al.) Okkelberg (1918) was not able to set up criteria by which the germ cells could be identified with any certainty in the cleavage or later states. He writes that all cells are more or less masked by yolk. However, he satisfied himself that he had properly identified the germ cells in the early stages of head elongation, when the embryo has a "comma-shaped" appearance when viewed from the side. Following his description: in E. lamottenii the mesoderm has already separated from the endoderm at the head end of the animal, but caudally the division line could not be distinguished between the 2 layers. The mesendoderm in the region extends dorsad as 2 ridges, one on either side of the nerve cord and notochord, and these are bounded externally by ectoderm. The cells of the mesendoderm vary in size, are irregular in shape, and most of them are filled with yolk. Numbers of larger, rounded cells can be distinguished from the foregoing, nevertheless; they are in the posteriormost fourth of the mesendodermal ridges. Okkelberg concluded that these were the germ cells, based on their apparent subsequent development.

B. Undifferentiated Stage

I have found what may be the precursor of the germ cells in the Maryland Brook Lamprey, in a larva of about 204 hours (laboratory culture at about 20° C.). This larva has the "comma" appearance already referred to, the serif of the comma being the head. The longest axis from the cervical curvature to the posterior caudal curve - the "rump" - measures about 1,150 µ in length. At this time isolated cells with a finely granulated cytoplasm are located in a tissue which is found between the outer ectoderm and the heavily laden yolk field and wherein in later stages both notochord and mesodermal structures are to be found (Fig. 5). For reasons which will be apparent, I cannot identify these cells with certainty. This tissue does not resemble endoderm; its yolk inclusions are smaller than those in that germinal layer and are not as crowded. The cells have cross-axes of 8.6 x 5.6 µ, and the nuclei are about 2 µ in diameter. They stain deeply with iron-alum hematoxylin. Such nuclei may be distinguished from yolk granules by their uniformly central position in the cell, when the area is scanned under low magnification, and by their relatively identical sizes. In some cells there are small cytoplasmic inclusions which may be yolk granules in the process of being absorbed. The larger yolk placques are not included in such cells. However, these cells are found in relatively the same place where Okkelberg discovered the germ cells in E. lamottenii.

In a later stage of head elongation (about 228 hours at ± 20° C.) where the cervical flexure-posterior curvature length measures about 1,700 µ (estimated total length; about 2½ mm.), a group of cells appear on the ventral face of the mesomere, in relatively the same area which later supports the gonadal strands. These cells have the same dimensions in the aggregate as those in the animal of 1,150 µ "rump" length.

Their cytoplasm is free from yolk material, and is very finely granular. The long axes of the cells are not quite parallel; if produced, they would converge in the animal's midline (Fig. 6). This group of cells directly abuts the yolky endoderm. The nuclei are likewise centrally placed, and are about 2 μ in diameter. If these are the germ cells, as I think they may well be, they lie in the region of the nephrotome.

In the hatched larva of 5.95 mm. slide length* small nests of reproductive cells are found lying between the postcardinal veins and the tubules of the excretory system. They are thus ventrolateral to these veins. They seem to begin just posterior to the junction of the pharynx with the intestine (Fig. 7) but are found only in the middle third of the animal. They are distinctly larger than cells of the adjacent tissue and have an ellipsoidal to pyriform shape. The cross-axes are 12.9 x 15.1 μ, with nuclei about 6.0 x 4.0 μ in the two diameters. The cytoplasm is somewhat more hyaline than that in other tissue cells, and exhibits a finely granular, even reticulate structure. The nuclear membrane stains more deeply, and very dark, contorted strands can be seen in the nucleus. The cellular membrane is well-defined and the cells are in loose contact, not appressed to one another. These small nests of approximately 6 to 12 cells are arranged in several separate clumps near the midline.

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*From the collection of W. H. Bayliff. The larva was hatched in his laboratory and was 7 mm. long at time of fixation. Adult lampreys have been taken by me from the small brook from which Mr. Bayliff procured mating pairs; I identify them as the Maryland Brook Lamprey. No other species of lamprey has been found in this particular brook, which is unnamed.

A 13.5 mm. (relaxed length) larva caught in Bear Branch July 21, measured 11.5 mm. on August 1, when it was fixed. Slide length was about 9.2 mm. Longitudinal serial sections of this animal show that the most anterior germ cells form a thin band lying in connective tissue between the dorsal aorta and the post-cardinal veins; they appear to begin about 250 μ posterior to the caudal tip of the liver. The germ cell clusters are still separate, one on each side of the dorsal aorta (Fig. 8). They are 15.5 x 9.5 μ in diameter, polygonal in shape from compression, and but few in each group. The nucleus is now large and is nearly spherical; it is about 4.5 μ in diameter. There is a distinctive but small nucleolus, somewhat eccentrically placed. The cytoplasm is quite clear, but shows a finely granular appearance under high powers. Its limiting membrane can be discerned with some difficulty. The nuclear membrane is sharply defined, and in the nucleus the stringy appearance provided by the chromonemata is plainly visible. The larger size, more or less round shape, more lightly stained appearance, and to some extent the appearance of the nucleolus distinguishes these cells from those about them.

To complement the observations made from longitudinal sections of the 13.5 mm. larva, a comparison has been made with observations of serial cross-sections in a larva 13.75 mm. long, cut at 8 μ (slide length, about 9 mm.) This animal was caught in Bear Branch July 21 and was fixed the following day. In these cross-sectinns the germ cells have been traced far forward to a point just posterior to the sinus venosus; here they lie in the connective tissue between the posterior wall of the sinus and the dorsal aorta. The cells occur, as has been stated, in clusters (Fig. 9), which are adjacent to the dorsal aorta and between it and the mesial surface of the endothelium of the post-cardinal veins. They extend posteriorly for about 2.8 mm. in interrupted strands on both sides of the dorsal aorta, and proceed down over the ventromesial face of the mesonephroi but external to the latter. The cross-axes of these reproductive cells come to about 12.9 x 11.2 µ. Their internal appearance is similar to that described under the 13.5 mm. larva; the chromonemata are clearly visible, and the nucleolus is present. Also, where chromonemata appear to meet, there are deeply staining granules of variable size. The finely granular material of the cytoplasm is in large part adjacent to or in contact with the nuclear membrane; and at one side there is a round clump of denser cytoplasm, in which there appears a deeply staining granule (sometimes there are 2). This may be a centrosphere with the included centriole or centrioles.

In the 17 mm. larva the reproductive cells are still in separate strands, and longitudinal sections show them in 2 rows extending posteriorly adjacent to the inner face of the post-cardinal veins. Cross sections of the 19 mm. larva exhibit the same picture. In both cases, the gonads extend posteriorly about 2/3 of the distance from the apex of the liver to the vent. They present quite the same appearance as described for the 13.5 and 13.75 mm. larva. Cells are variable in shape; if they are cylindrical, they may be 21.5 x 8.6 µ (Fig. 10). Discrete groups of cells enclosed by epithelia are found in a continuous band in the 20 mm. ammocoete. The cells are appressed and the nuclei are large: about 5.5 µ in diameter.

C. Development of the Oögonium

The germ cells are sexually differentiating in the late summer of the first year. A larva caught August 27 measured 37 mm. when the length was taken on the following day. It was killed and fixed September 17. By the last date the appressed oögonium had the average dimensions of 73.6 x 63.6 mm. (Fig. 11). The cytoplasm is divided into 2 well-defined areas: a hyaline, ovaloid central area about 25.8 µ in diameter in which a darkly staining round body of 8.6 µ in size is eccentrically located; and a very finely granular field which is destined to be the yolk area. The granules stain deeply with iron-alum hematoxylin. There is apparently a sharp separation between the granular and hyaline areas; but an actual membrane appears to be absent. The circumscribed hyaline area has been identified in other brook lampreys as the nucleus, but development demonstrates that in the Maryland Brook Lamprey it is hyaline cytoplasm. It follows then that the smaller, darkly stained round body is the nucleus, and not a nucleolus. It is emphasized that if the hyaline area were considered to be the nucleus, it would require that the germinal vesicle must have grown many times larger than the primordial germ cell, and that the nucleolus has attained the size of the original nucleus. The size of the meiotic equatorial plate at the first maturation division and the subsequent dimensions of the oöcyte nucleus which appears in the same hyaline field, is considered to support this writer's conclusion.

At the same time there appear in this ovary nests of cells (Fig. 12) which very strongly resemble those found in the 20 mm. larva. It seems to be assured, then, that the transitional stage has been found in those cells destined to become ova. The relatively undeveloped cells have a more deeply staining cytoplasm than those of the 20 mm. stage, and the nucleolus does not stain so deeply. They may be regressive.

The intermediate stage between primary, undifferentiated cell types and the characteristic oogonium will be a subject for further study. From the appearance of certain cell groups in present material, however, the large size of this sex cell seems to be attained by an unusual process. It seems to be that most, if not all of the cells of the unit follicle contribute to the ultimate oögonium. These cells enlarge considerably, and their cytoplasm becomes very granular. The cellular membranes between adjacent cells disappear, while at the same time the vitelline membrane comes into view. The fate of the nuclei concerned is uncertain, but they endure for an undetermined period of time after the walls of the contributing cells have been dissolved (Fig. 13). It will be observed from the illustration that what I take to be the original nuclei are still present. A later stage shows them in a very reduced state (Fig. 14). At this time the granular cytoplasm stains more deeply than the cells in other stages.

D. Development of the Spermatogonium

A larva 46 mm. long caught on August 27 (the same day that the 37 mm. differentiating female animal was obtained) was also killed and fixed on September 17. The sex cells are located in tubule-like groups; they are surrounded by a very thin epithelium and there is a lumen in the center of the group (Fig. 15). From an examination of the dissected testis of a mature animal, it is evident that this is the beginning of the lobular system characteristic of the adult male reproductive organ. It is also certain that sexual differentiation has begun, as in the 37 mm. larva (cf.) the ova are now readily distinguishable. The cells are small, with scant cytoplasm and large nuclei. The last are quite spherical and are about 6 μ in diameter in a cell about 8 μ long. The nucleus is provided with a small nucleolus and exhibits a very reticulate, hyaline interior, the chromonemata being readily discernible. Cell boundaries, on the other hand, are difficult to distinguish.

2. Second Year Larvae

. The Oögonium

The female larva of 76½ mm. length (caught August 27, fixed September 17) has the single ovary in the midline between the mesonephroi. The oögonia have grown considerably; they are about 130 µ in diameter. The clear cytoplasmic area has a diameter of 50 µ, and it possesses a finely granular reticulum. The nucleus has attained a diameter of nearly 13 µ, and stains deeply. The yolk area of the cytoplasm is extremely granular, and the yolk granules stain deeply (Fig.16). In larvae of 82 mm. length the ova has cross-axes of 150 x 135 µ.

The clear hyaline cytoplasm is sharply delimited in oögonia of a larva of 86½ mm. length caught and fixed on February 2, towards the close of the second year. It has cross-axes of 46 x 70 µ in an agg of 164 x 124 µ, and is provided with a nucleus measuring 17 µ in diameter. A larva of 101 mm. length caught and fixed on the same day had sex cells with practically the same dimensions and very much the same appearance (Fig. 17). In one favorably stained nucleus of an oögonium of a 101 mm. second year larva of October 27 (fixed the same day) a very distinct internal organization may be discerned (Fig. 18). Contained within the karyoplasm of the nucleus are found a series of oval to round bodies of varying size (from 6.5 to 1.0 µ in diameter). In the center of each of these is a more deeply staining granule or clump of granules. It is suggested that these clearly marked boundaries about the granules possibly may be borders of matrices about the individual chromonemata.

B. The Spermatogonium

In a 53½ mm. larva (caught August 13, killed and fixed September 17), the tubules are solidly packed with the rapidly dividing spermatogonia. The sex cells and their nuclei are very much the same size as that given under the first year larva of 46 mm. length. Mitotic figures show almost diagrammatic equatorial plates with typical spindle fibers; centrioles could not be seen. The numerous chromosomes are extremely small, and the entire equatorial plate has a diameter of not more than 7 to 8 μ; the individual chromosomes stain intensely with iron-alum hematoxylin, and none of the chromosomes achieves a length of 1 μ (Fig. 19).

3. Third Year Larvae

A. The Oögonium and the Oöcytes

Except for growth, the oögonium does not change materially in appearance until the fall. In October the germ cell of the larva of 99 mm. length has a variable but somewhat ellipsoidal form; the average diameter of a circle of equivalent area in the plane of section would be about

385 μ. On October 13 (animals caught and fixed the same day) the future single egg exhibits a deutoplasm in which the larger yolk granules have attained dimensions of about 4.5 x 5.3 μ; and these begin to resemble the yolk of the mature egg. The granules are evenly distributed throughout the egg except at the periphery, where a circumferential series of small vesicles begins to appear (Fig. 20). These vesicles are about 8 μ in diameter and foreshadow the advent of maturation. There are also small, isolated clumps of yolk granules which stain very much more deeply. The significance of this is not understood.

The formerly hyaline area of the cytoplasm has now become more finely granular and stains more intensely. This erstwhile clear area, -- which for convenience will be termed the paranuclear cytoplasmic field -- has cross-axes of about 65 x 104 μ. The darkly staining nucleus has a diameter of 20 μ

At this stage the nucleus and its circumjacent cytoplasmic field is located in many cases closely to one pole of the somewhat oval cell. The narrow layer of deutoplasm thus created between the lighter cytoplasm and the vitelline membrane undergoes a transformation. This "polar" cytoplasm consists of a denser, more finely granulated material, which appears as a curved band in cross-section (Fig. 21). It is always associated with the close proximity of the nucleus to the vitelline membrane. In it are little vesicular pockets which appear to contain larger granules similar to those of the rest of the yolk cytoplasm.

As the nucleus with its surrounding more or less hyaline cytoplasm approaches the vitelline membrane at the animal pole, the vesicles about the periphery become more numerous. There is at the same time a redistribution of the yolk granules periphered, leaving only the finest particles centrally. During this time the relatively yolk-free cytoplasm about the nucleus has shrunk to ± 20 x 15 μ, and it presents a deeply "scalloped" face to the yolk cytoplasm, because of vesicles formed at the division line between the 2 kinds of cytoplasm. The nucleus has now approached the polar edge of the clearer cytoplasm and stains much less intensely. It is still 20 μ in diameter. The internal structures which are possibly chromosomal matrices are clearly visible; in one nucleus a figure-8 configuration was observed. This may have arisen either from constriction in one, or incomplete fusion of 2.

As the primary oocyte approaches the first maturation division, the vesicles around the periphery are augmented by new ones appearing throughout the yolk. The paranuclear cytoplasmic field is compact and somewhat "pill-shaped". It now stains more deeply with the iron-alum hematoxylin than the deutoplasm, and has arrived to within 60 μ of the egg membrane. Next to the nucleus, and between it and the vitelline membrane, there arises another hyaline, cap-like cytoplasmic area. This is called by German writers the "Deckel". This is derived apparently from the deutoplasm between the paranuclear cytoplasmic field and the vitelline membrane. In fact, in many sections there appears to be a sharp cleft here between the two fields where they meet. The nucleus is found at the edge of the paranuclear cytoplasmic field in any location (Fig. 22).

All of the foregoing observations were made on oöcytes of a larva of about 100 mm. length taken on October 13 and killed and fixed on October 18. 5 days later another female animal was killed, and fixed by the drying and embedding method developed by William E. Maloney and the writer. In the primary oöcytes from this ammocoete, therefore, the chemical constitution of the egg will not have been altered by any reaction with the ions present in the aqueous dichromate-acetic acid fixatives used elsewhere. The only reactants could be the agents used in the staining and mounting processes. The appearance of the sex cell under the microscope differs only in minor aspects from that hereinbefore described; this gives assurance that both techniques are relatively comparable.

In this maturing lamprey the oöcyte now has the characteristically compressed, somewhat polygonal outline of the immature egg, and if reduced to circular dimensions it would have a diameter of about 600 μ. The paranuclear cytoplasmic field is crescentic to lenticular in sectional outline, with the nearer side within 60 μ of the vitelline membrane, and more or less parallel to the latter. This area is about 120 μ long and 60 μ wide at the middle point. The deutoplasmic field stains quite intensely with iron-alum hematoxylin, and is quite vacuolate throughout. The cytolymph does not stain at all, differing in this respect from material fixed with the Liquid of Tellyesniczky; such material by contrast shows a light violet-gray structure. The paranuclear cytoplasmic field in the present case also refuses to stain, and appears as a coarse, yellow reticulum. Opposite this clear cytoplasmic field and at the outermost periphery of the oöcyte there is a cap of hyaline cytoplasm, which is a new feature in the maturing oöcyte. This cap has a thickness at its widest part of 20 µ.

An examination of the equatorial plate at the beginning of anaphase in the foregoing material, discloses stocky, very short chromosomes. They are scattered in an equatorial plate about 16 µ across. The largest chromosomes do not exceed 3.5 µ in length and some of them are less than 1 µ in size (Fig. 23). The cytology of this process is still under investigation.

On February 2 a metamorphosing 118 mm. long female lamprey was obtained. It was narcotized February 7, and the ovary dissected out and fixed. The secondary oöcytes had now achieved dimensions of about 800 x 1,050 µ (Fig. 24). They present otherwise an appearance very similar to that of the primary oöcytes. The nucleus is reconstituted. The yolk granules are coarsest in the center of the egg, and here they attain a size of 8 x 4 µ; they are more or less disk-shaped. Towards one end or pole of the somewhat ellipsoidal cell they become much smaller. Near the vitelline membrane there is in the yolk field a highly vacuolate periphery, giving the deutoplasm here a frothy aspect. The somewhat spherical hyaline field lies about 1/3 nearer one pole; in this area existing granules are much smaller. This hyaline cytoplasm sends many reticulate extensions towards the pole. Eventually the clear polar cytoplasmic cap is connected with these cytoplasmic extensions. The haploid nucleus at present lies in the deeper, internal part of the hyaline field, and at this stage it stains very little. It is quite spherical and measures 17 µ in diameter. Otherwise it has very much the same internal appearance as it had before the first reduction division. My present material does not disclose the first polar body.

In the mature female lamprey of April 28 the secondary oöcyte has a nearly spherical contour and a diameter of about 925 µ. This is the "egg" which is visible through the abdominal wall of the gravid female. This oöcyte has a plasma membrane which (1) stains moderately with iron-alum hematoxylin, and (2) is less than 1 µ in thickness (Fig. 25). Under ordinary circumstances the vitelline membrane closely invests the plasma membrane, but it is not attached to it, as occasional warping shows. This outer coat consists of at least 2 layers. The innermost one stains moderately and is about 3 µ thick. The apparent external layer stains less readily or very little, and is about 8 µ in width. Outside of these layers there seems to be an extremely fine deposit of almost submicroscopic fineness, evident when the membrane is torn. In the living egg the vitelline membrane has some elasticity; but its tensile strength is quite weak, and it ruptures easily.

There is a more or less centrally placed, rather hyaline core of cytoplasm, filled with very fine granules of 2 μ in size or less. This leads to a quite yolk-free polar cap which, as deduced from subsequent development, is located in the vicinity of the animal pole. The nucleus lies in the central part of the egg in this hyaline core (Fig. 25) and is slightly oval in contour; it has cross-axes of about 16 x 18 μ. The nuclear membrane encloses a remarkably clear interior, wherein the chromonemata can be seen with ease; and blackly staining, granular lumps can be seen as though threaded on these strands. The yolk material now stains densely with iron-alum hematoxylin; these granules may reach 10 μ in length, are flattened, and are more or less oval. They decrease greatly in size toward the animal pole, where they may be barely resolvable. Between this stage of the secondary oöcyte of April 28 and the time of spawning, the nucleus proceeds to form the metaphase plate of the second meiotic division.*

B. The Spermatogonium and Its Descendants

The first maturation division has begun in spermatogonia of the 30-month old lamprey of November 1. In a male of 116½ mm. length killed and fixed that day all premeiotic stages may be observed, and the equatorial plates are clear-cut and almost diagrammatic.

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*This date of April 28 applies only in this instance. The Maryland Brook Lamprey has spawned in the laboratory about April 2 (temperature = 16° C. and more). Obviously this second reduction division took place on or shortly before April 2.

i. The primary spermatogonium at the beginning of meiotic prophase:

The germ cells are tightly packed in the lobules, and where cell boundaries may be seen, they indicate that the spermatogonia are compressed. The cells consequently are irregularly polygonal in shape and have diameters of about 8 to 9 μ. The nucleus is quite spherical, and the nuclear membrane stains well (Fig. 26); its diameter is approximately 6 μ. In the nucleus there is an eccentrically placed nucleolus, and the very clear karyolymph surrounds the contorted chromonemata. Because of the extremely small size of this karyokinetic system, many of the details cannot be resolved with the light compound microscope. I am, therefore, unable to determine whether these chromonemata are single or double; but they are very attenuated and evenly dispersed. Some of the nuclei may well be in the leptotene stage (see below).

ii. Meiotic prophase I:

Before describing stages in the prophase of the first reduction division, I must state that I have not had the opportunity to investigate and determine whether or not the generally accepted accounts of this prophase are applicable in this instance. Nevertheless, configurations are found which can be fitted into most of the stages as described, for example, by Sharp (1943), De Robertis, Nowinski and Saez (1949) and Rugh (1951). With the foregoing in mind, therefore, this account will follow those of the authors just referred to.

The leptotene stage cannot readily be distinguished from that of the premeiotic spermatogonium.

The zygotene stage (Fig. 27) is believed to be identifiable from the somewhat close association of the chromonemata in a tangled weft about the nucleolus. There is, however, no characteristic "bouquet" stage as occurs in some organisms. Reinforcing this observation is the fact that the chromonemata are quite pyknotic and appear to be thicker, as though they had been doubled.

In the next stage the pachytene chromonemata develop their matrices and the resultant chromosomal units stain darkly (Fig. 28). A criterion of this state which helps in its determination seems to be the clumping of at least some of the chromosomes about the dwindling nucleolus. The chromatids have a sticky appearance.

The diplotene stage is one of those most readily established. By this time the nucleolus has disappeared, and the thick, heavily stained chromatids in the process of terminalization are distributed over the inner face of the nuclear membrane. The result is a coarse, black, net-like spherical configuration, with a hollow center. These separate in the next stage.

In diakinesis individual tetrads can be distinguished and in one case observed they were still distributed in a hollow sphere, even though the nuclear membrane had vanished

(Fig. 29). These then migrate to the equatorial plane of the cell.

iii. Meiosis I. The metaphase:

From the standpoint of an ideal representation, the metaphase equatorial plate is all that the researcher could wish for, with one important exception. It is beautifully delimited; the so-called spindle fibers are easily discerned; the spindle figure is symmetrical, and when viewed from the side is shaped like the ace of diamonds with the very plane equatorial plate at the center of the shorter axis. The spindle fibers terminate in a sharp point (sometimes 2) at their poles. Centrospheres seem to be lacking, and there is no aster. The one difficulty is that these chromosomes are so closely associated during this stage that in my present material they cannot be counted with any assurance (Figs. 30 and 31).

iv. The Metabolic Stage (Interkinesis). The secondary spermatocyte:

This stage has been examined in a maturing male obtained on July 5 when it was 95 mm. long, and retained in the laboratory until December 15 of the same year, when the testis was removed and fixed. The secondary spermatocytes are beginning to round off and to separate from one another; free cells are spherical, with a more or less equally round nucleus, and they do not entirely fill the lobule. Diameters are: cell, about 8.6 μ; nucleus, about 5.6 μ (Fig. 32). Within the nucleus there is a dark, irregularly shaped, deeply staining granule, somewhat less than 1 μ across, and from which in many cases the chromonematic threads seem to radiate. This granule may be found anywhere within the nucleus; it is often found against the nuclear membrane. The nucleus has a denser appearance than it had in the primary spermatocyte.

v. Meiosis II:

The equatorial plates of the second reduction division have been found in a metamorphosing male lamprey 101 mm. long, caught on February 20; the testis was fixed the next day. The lobules are somewhat loosely filled with germ cells which are variously in interkinesis, prophase and meiosis (Figs. 33, 34). There seems to be no special distribution pattern of these stages in the lobule, except that in one lobule most of the cells tend to be in only one of the stages.

At this time cells are found which are quite similar to those described under the metabolic stage. Differences are: (a) the karyoplasm now is much clearer, even hyaline; (b) the large internal granule hitherto found has decreased in size or has vanished; and (c) the nucleus is smaller (about 5 μ).

In other lobules in which there are few equatorial plates in proportion to the total number of cells in lobular section, other nuclear patterns are found. It is my opinion that, based on spatial contiguity with both metaphase and metabolic stages, these other patterns are those of the second meiotic prophase. This furthermore seems to be confirmed upon comparison with an obviously early post-meiotic development described under vi. (Spermiogenesis). The 2 stages are very different in appearance.

During this prophase the nucleus expands considerably (diameter = 7 μ). The chromatinic elements are distributed on the inner face of the nuclear membrane, except for either (a) a viscid appearing, thin, granular band running from wall to opposite wall, or (b) an irregularly shaped clump at one side. Chromonemata may or may not be apparent in these internal structures. The cytoplasm appears to be denser, and in many instances most of the chromatic material of the nucleus progressively clumps together at one side of the nucleus. Now the cell elongates, the nuclear membrane disappears and chromosomes are organized from the clumped chromatids. Then these migrate to the equator of a spindle which is so closely invested by the cell membrane that the cell itself seems to be merely the equatorial plate and the achromatic figure. At this time cell dimensions are 12.9 x 6.5 μ (Fig. 34).

The metaphase plate has the conventional configuration, and spindle fibers are visible. The cytoplasm at the poles is denser than elsewhere; there seems to be a small centrosphere, but I could not find a centriole. The sticky-looking chromosomes are extremely difficult to count because of their tendency to associate closely, and a study of chromosome number is reserved for the future. At disjunction the 2 haploid complements are at first almost parallel to one another but the migrating sets become cone-shaped in late anaphase. On one side of the spindle 1 chromosome is seen to advance to the pole in advance of the others; this is perhaps a sex-determining chromosome.

The equator of the cell becomes narrower as the haploid chromosomes separate in anaphase. Finally a slight equatorial furrow becomes evident, and a flat cross-septum grows, separating the daughter cells. The nuclei of the incipient spermatids have diameters of about 3 µ in cells roughly 5 µ in size; and the karyoplasm is dense.

At this point it should be stated that the writer has found meiosis II taking place as early as February 7, or 2 weeks earlier than in the example just given. Possibly laboratory temperatures of 18 to 22° C. may have stimulated the development of the animal during a 5 day sojourn in the aquarium from February 2, when it was obtained.

vi. Spermiogenesis:

The development of the spermatid was found to have started in a lamprey of 110 mm. length caught on February 21, and killed March 20. In this case the month's maintenance in an aquarium at laboratory temperatures may not have influenced development; another animal caught on March 20 and killed the next day, contained spermatids in practically the same stage.

I start with the recently divided secondary spermatocyte where the daughter cells (spermatids) are still in contact.

However, the cells have become quite rounded. The nucleus is now about 4.5 μ wide in a cell of 7.5 μ; the chromatin is deeply stained in a clear karyolymph. The chromosomal material is mainly disposed on the inner face of the nuclear membrane. Externally, at one side of the nucleus and almost in contact with it, there are 2 fine dots. These may well be derived from a centriole.

Shortly after separation of these daughter cells, their nuclei become much more pyknotic; in fact they stain so deeply that they are nearly opaque (Fig. 35). The nucleus is displaced to one side of the cell during this change and becomes oval. The 2 external granules just referred to are found near the nuclear membrane in the larger cytoplasmic area, near a little depression in the nuclear wall. The cytoplasm grows; it increases the cell diameter to 8 μ. The 2 granules move to about 2 μ from the nucleus, and are seen to be surrounded by a halo-like lighter zone. They are now so arranged that a line drawn through both would coincide with the shorter axis of the nucleus; and they are about 0.3 μ apart. The granules stain darkly, and from the most distad one an extremely fine fibril is observed to traverse the cytoplasm to the edge of the cell, directly away from the nucleus (Fig. 36).

Following this, the nucleus again becomes spherical and apparently shrinks (4 μ). It stains uniformly and opaquely; but the gradient of staining intensity deepens gradually toward the edge of the disk as viewed. This indicates that the chromatinic material is for the moment dispersed more smoothly over the inner face of the nuclear membrane. The distad cytoplasmic granule has migrated to the edge of the cell farthest from the nucleus, and a free filament is growing from it. A prominent additional granule comes to view now for the first time; this may be the paranucleus (Nebenkern, chondriome).

The next stage in development is elucidated from tissues taken and killed March 20, from a male lamprey of 99½ mm. length on February 2. Striking changes are taking place at this time. After the pyknotic contraction period undergone by the nucleus just described, it reverts again to a more hyaline condition. In plane section the spermatid begins to resemble a round-headed kite, with the nucleus in the upper half-circle (Fig. 37). Below, facing the "tail", the nucleus is flattened. Resting against this flattened nuclear membrane there are at least 2 granules, slightly separated from each other. A fine filament leads from each of these 2 granules to another one more distad, creating a triangle in which the wall of the nucleus forms the base; inside of the triangle there is a very dense material. Just beyond the outer apex of the triangle there is a larger granule, from which the tail filament originates. There seems to be a connection between the blepharoplast-like distal granule and this apex of the triangle. At this stage I have been unable to identify a paranucleus. The cytoplasm tapers for a very considerable length along the tail filament; the cell has therefore a length of 14 μ and more. None of these cells or the later stages which may coexist with them are to be found in any significant arrangement within the lobule.

The next step in this sequence is that the nucleus begins to elongate, and the latter assumes a characteristic "teardrop" shape, with some diminution in cross diameter (to 4 and 3 μ). The point of the "drop" extends toward the tail process (Fig. 38). At this time there can be seen in the nucleus a fine fibril which extends from the "centriolar" apparatus toward what may be properly termed the acropylar end. In this acropylar region, and apparently just outside of the nuclear membrane, there come into view a few blackly staining granules. These granules are found in contact with the nuclear membrane at first on one side; they move on the nuclear face toward the acropylar central point and coalesce. This seems to be the acrosome. I have not been able to demonstrate any association with a Golgi Apparatus.

The nucleus progressively elongates (to 7.2 μ) and again stains opaquely. At this point a series of fine fibrils connects the posterior point of the nucleus with the "centriolar" granules, and these seem to be associated with the middle piece of the developing spermatozoon. At the acropylar point there is an intensely staining, very dark granule.

On March 21 the maturing spermatozoon has begun to assume the habit of the ripe male sex cell. The cytoplasm about the nucleus has become so thin that it cannot be distinguished. The rest of the cytoplasm has migrated toward the tail, and then it seems to disappear. The mode of this disappearance has not been resolved.

The nucleus now becomes the head of the spermatozoon. It becomes pyriform in shape, and then elongates towards the acropylar end. Internally, granules and ragged patches of chromatin may be observed until maturity, when it stains so deeply that very little of this detail can be seen (Fig. 39). There is a very small granule persisting at the acropyle, from which an extremely thin filament grows; this will be referred to under vii. (the Spermatozoon). The middle piece elongates, and divides into a spirally twisted anterior and a cylindrical posterior part. At the beginning the middle piece is about 3 μ long, and terminates in a black point (perhaps 2 points). From this the tail filament extends posteriorly.

vii. The Spermatozoon:

The adult spermatozoon has a head piece (nucleus), head filament, middle piece, and tail filament (Fig. 40). Observations have been made on spermatozoa withdrawn from the body cavity by pipette, and under the following conditions: (a) in the fresh state, suspended in water; (b) as stained by aqueous Gentian violet and methylene blue; (c) in aceto-carmine and (d) as presented by the general methods already described. Fresh material perhaps may be active when ejaculated at the time of fertilization; but by the time sperm can be withdrawn from the animal's body cavity, suspended, placed on a slide and brought into focus (about 15 seconds) they are immobile. In concavity-slide preparations suspended in water the filaments can be seen very readily. Dimensions are, approximately:

		Micra	
		Length	Width
Head filament		13	*
Head (nucleus)		12 to 13	1.5
Spiral part	) Middle piece	25	.5 to 0.8
Cylindrical part		15	-0.4
Tail filament		160 to 200 and over	
	Total length	225 to 266	

(1) The head is lancet-shaped, and slightly curved. It tapers to a point anteriorly. The nucleus stains deeply with iron-alum hematoxylin, although clear, vesicular-shaped areas can be seen. The head terminates in a very small acropylar knob from which extends

(2) The head filament. This is best seen in Gentian-violet stained material. It is exceedingly fine, and can be seen in living material only under favorable conditions. The apparent continuation of the head filament traverses the nucleus longitudinally posterad, to terminate in a granule-like knob at the beginning of

(3) The spiral part of the middle piece. This consists of a very finely cylindrical core about which a spirally wound ribbon-shaped structure undulates in about 6 turns. At the caudal end of this part there is a shaft which terminates in 2 granules. Beyond this extends

(4) The tail filament. This is not anteriorly as thin as the head filament. It appears to taper off to a fineness

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*Too thin to measure.

which cannot be resolved with available equipment.

The spermatozoon is extremely fragile. Rapid pipetting will fragment it; and aceto-carmine dissolves the filaments. In other media these cells form tangled wefts. They are particularly prone to coil on themselves, and look like signet rings as a result.

CHAPTER IV

FERTILIZATION AND CLEAVAGE

1. Fertilization

A. Changes in the Oöcyte During the Spawning Period

Following the prophase-like configuration described on pp. 46 and 47, it is found that the nucleus migrates to the area of the hyaline polar plasm where it comes to lie immediately under the plasma membrane. The coarser yolk granules are distributed in the vegetal hemisphere of the egg, but finer deutoplasm has infiltrated under the plasma membrane beyond the equator for perhaps 30°. The foamy appearance so apparent at Meiosis I is still retained, but the vesicles are small and are scattered just under the plasma membrane.

Some of the ensuing developments now to be described are inferred from observations made on the behavior of the fertilized egg collected within 15 minutes of spawning; slide sections of stages just prior to Meiosis II in the oocyte have not yet been available.

No polar body has ever been observed associated with an unfertilized egg. Many slide sections of such eggs, fresh, moribund and in various stages of dissolution, have been scrutinized; in these, likewise, no second polar body has ever been found. Conversely, when the second polar body has been observed, the egg proceeds to cleave. From the foregoing I am of the opinion that this oöcyte, just as that of other vertebrates, forms the metaphase plate of Meiosis II parallel to the plasma membrane. The metaphase then stays in arrest until the entrance of the spermatozoon, whereupon it completes the meiosis. The extrusion of the second polar body has been observed both from slide material (Fig.41) and in the living zygote.

The newly fertilized egg is very nearly spherical in contour, except for a flattening in the future animal pole. It is a very light buff-colored to greenish ball, almost white, and roughly 1 mm. in diameter. The area of the animal pole is cream-colored and has a white fovea or depression more or less at its center; the later constitutes the central part of the polar cytoplasmic cap wherein the nucleus would be found. The vegetal hemisphere, as well as a band of variable width above the equator of the egg (towards the animal pole) is an extremely light-green -- what I would call an apple-green. This color deepens at the vegetal pole, and fades above the equator; the border between the 2 areas is diffuse. To some extent the slight mellowness of appearance is contributed by the vitelline membrane, for the contents of freshly ruptured eggs are lighter in tone (Fig. 42).

A gelatinous envelope surrounds the egg, and upon contact with water expands to about 250 µ in thickness. It consists of at least 3 layers: (a) An outer, translucent, thin and viscid layer of variable width, to which small particles of gravel adhere with great tenacity. It behaves much as though it were a mucin. Like a slime, this layer oozes over the fine particles adherent to the envelope; and when the egg is detached from this granular layer, the particles still clump. Fine strands of this material can be seen stretching between the sand grains when the latter are pulled apart. (b) The middle layer, which constitutes most of the egg capsule, is quite transparent. It has an outer wall which is elastic and tough, and which may be a precipitation membrane. It turns opaque in dead eggs. (c) An internal layer, which is very thin and invests the vitelline membrane.

B. Syngamy

The spermatozoon, either from perhaps its own transient activity, the directive force of ejaculation, or the great number of spermatozoa expelled to make contact more probable, comes to lie on the outer viscid coating of the egg. Contact with an egg is possibly enhanced by the length of the head and tail filaments; but eggs frequently are layed which do not become fertilized. There is no micropyle; the male sex cell travels through the vitelline membrane leaving it in a slightly undulating or helical path (Fig. 43). At the instant the spermatozoon touches the plasma membrane, or very shortly thereafter, this latter membrane appears to dissolve or rupture at the point of contact; and a small, conical elevation of the egg cytoplasm is raised about the point of entrance (Fig. 44). The spermatozoon loses its head and tail filaments in the act of entering the vitelline membrane. There is apparently no specificity on the part of this outer membrane as to the number of spermatozoa which may go through it; however, only 1 male cell seems to be permitted to pass through the plasma membrane. It is possible that the successful spermatozoon may enter the egg anywhere; but in 3 observed cases, the male pronucleus is found in the incipient animal hemisphere. Supernumerary spermatozoa come to rest externally on the plasma membrane and degenerate.

At the time that the spermatozoon enters the ovum, the latter responds by immediately undergoing a number of changes: (a) the cell shrinks in volume slightly, and retracts from the vitelline membrane. The resulting zona pellucida shows reticulate, hyaline strands coursing irregularly from the plasma membrane to the inner face of the vitelline membrane. The latter is warped and buckled in sectioned material as a result. (b) Meiosis II in the egg is completed, and 1 of the haplonts is split off as the second polar body. (c) The hyaline cytoplasm undergoes a change in position. The polar cap becomes superficially thinner; most of its cytoplasm appears to collect centrally near the animal pole, and assumes a lenticulate form. However, at this time there is no instantaneous peripheral migration of the cytoplasm as has been reported in L. fluviatilis (Lönnberg et al., 1924). (d) The axial strand becomes thinner, more extended and eccentrically placed, and is more sharply delimited from the deutoplasm.

Shortly upon penetration of the egg, the head of the spermatozoon begins to transform. The lancet-shaped nucleus shortens somewhat and turns end for end so that its former posterior end is directed, as well as the longitudinal axis of the still characteristically shaped head, towards the center of the egg. Here a centriole appears proximately, surrounded by a very finely radiate aster. The cytoplasm of the egg circumjacent to the sperm-nuclear area is stained as though some of the material which had formed part of the spermatozoal head was breaking down. The area outlined by the head is about 9 x 12 µ, showing that it is beginning to alter to the form next described (Fig. 45).

In its progress along the copulatory path the now male pronucleus seems to leave a very narrow, rather torturous and hyaline path in the deutoplasm. After it has traversed a distance of 50 µ into the ovum it creates a translucent, spherical, relatively granule-free area in the finer deutoplasm about 22 µ in diameter. Very small chromatinic units, presumably the chromosomes, can be seen radially distributed throughout this hyaline body. No nuclear membrane can be observed at this time.

There are indications, correlated from the presentations in a number of slides, that the male pronucleus migrates toward the axial strand, while the female pronucleus moves, but far more slowly, towards the upper part of it. The reason for the uncertainty of identification at this time is: the chromosomal elements stain faintly and in the female pronucleus at any rate, appear to become very reticulate. The next certain appearance of the pronuclei (as I have been able to observe) shows them in the upper polar cytoplasmic area, at the animal pole, about 5 μ below the plasma membrane (Fig. 46). They have oval outlines, cross-axes of about 12 x 8 μ, appear to be denser than the cytoplasm, and have a finely reticulate internal structure. One of them appears to have a centriole, surrounded by a small halo. I suppose that this is the male pronucleus.

By this time there has occurred a displacement of the deutoplasm. The finer yolk granules are uniformly distributed over the periphery of the egg, and the coarsest deutoplasm has been relegated to a nearly central area. Here the largest yolk granules now assume a sticky appearance, and stain much less deeply. Sections support the conclusion that a wave of the finer deutoplasm has eddied down on one side of the egg as shown in the diagram. Undoubtedly the pronuclei now approach one another, and resolve themselves into kinetic figures in preparation for the first cleavage metaphase. Externally this manifests itself by 2 pimple-like elevations in the plasma membrane, with a fine, hyaline line appearing transversally in the opaque white fovea between them. The elevations are probably to be associated with the confluence of the cytoplasmic material about the cytasters of the first cleavage.

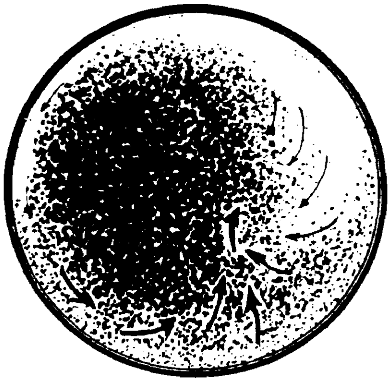

DIAGRAM SHOWING EDDY
OF FINER DEUTOPLASM

The total elapsed time between oviposition and the beginning of the first cleavage is at least 6 hours (18° to 22°C.), probably about 7, perhaps more. Endeavors to be more precise by interrupting the mating lampreys immediately following a spawning act leads only to an hour-long fright reaction. Repeated interruptions cause an indefinite cessation of activity. One would almost believe that the animals know when they are alone; not until I had retired (about 4 a.m.) on 2 occasions, did cyclostomal love-making then proceed with ardor the remainder of the night, judging from results the next morning, i.e., a large number of eggs were layed and fertilized.

2. Cleavage in General

All cleavages are holoblastic, as in other lampreys. The distribution of yolk is such that beginning with the third division, cleavages are unequal, with the production of micromeres and macromeres as will be described.

3. First Cleavage

A. External Morphology

Incipient to cleavage, the 2 little protuberances on the surface of the egg at the animal pole become more prominent. Between them the hyaline streak sinks down, and a crease indicates the location of the developing septum between the first 2 cells of the new embryo. This cleavage furrow lies at right angles to a line drawn through both of the initial prominences, and cuts the zygote vertically. Ideally it coincides also with the polar axis of the egg; but occasionally it is obviously paramedial, or more rarely tilted out of the vertical. This is evident in the latter case because the light green area of the egg then is divided unequally by the cleavage plane.

The first division septum (Fig. 47) begins to develop very slowly. After a variable period of time, roughly 90 minutes or more at 22° C., it subtends an angle of about 60° on each side of the animal pole. The furrow now becomes very deep, approximately 100 μ from the original curvature of the undivided zygote. In 30 minutes more (about 2 hours in all), the cleavage is completed. The embryo now resembles two smooth, old-fashioned, stemless china doorknobs, apposed base to base, and within the transparent vitelline membrane. The completed first cleavage endures for 35 to 40 minutes at 22° C.Toward the end of this period the strongly incurved walls of the cleavage furrow are somewhat flattened, apparently because tensions arising in kinetic phases of the first mitosis are relaxed by the accomplishment of the latter. As a consequence, the 2-celled embryo assumes a more spherical form, and the furrow where the cleavage plane is tangent to the outer surface becomes shallower.

B. Internal Morphology

The embryo at the end of first cleavage has cross-axes of about 977 x 853 μ. The cleavage furrows are about 75 μ deep. Each blastomere has its own internal cell wall and the surfaces of the two cells are adherent in a peripheral circular ring about 260 μ wide. Discounting the depth of the furrow, this leaves a cleft-like space between the two cells about 300 μ long and centrally about 40 μ wide (Fig. 48). This cleft is the beginning of the blastocoel. The hyaline cytoplasm is now distributed about each nucleus and in turn it is partly surrounded by the finer deutoplasm, which is found in the upper part of the cell. A remnant of the polar cytoplasmic cap persists at the animal pole. The cleft between the two blastomeres bisects the very coarse, sticky, granular area of the deutoplasm which has not, apparently, changed in relative position. External to this area, and in the remainder of the vegetal hemisphere, the large, deeply staining yolk placques are homogeneously distributed. The finer granules once observed in a peripheral band universally in the vegetal hemisphere in the early zygote, are now absent from this region.

4. Second Cleavage

A. External Morphology

The second cleavage is chiefly meridional and at right angles to the first. While both primary blastomeres initiate division at about the same time, situations have been seen where the second cleavage was asynchronous; indeed, one blastomere may complete division before the other starts. The planes of the cleavage in the two blastomeres may be in the same straight line, but more often they are offset as viewed from one of the poles (Fig. 49). The second cleavage starts at the animal pole or near it, and causes a furrow to appear at that place tangent to the septum of the first cleavage. This crease courses mesially down the side of the blastomere, and deepens progressively as it does so. In other respects the behavior of the furrow is very similar to that described for the first cleavage. The crease flattens, and the original spherical contour is more or less approached.

Toward the middle of the four-celled metabolic stage a characteristic event takes place, which is destined also to be reduplicated in at least the next 3 cleavages. Where the 4 cells meet superficially at the poles, they retract from one another and leave a small cavity between them. By the end of interkinesis they have again rejoined at the surface; nevertheless sections show that internally the cavity is often partly retained as a cleft between the cells.

From 6 sets of observations, 4 at 22° C. and 2 at 27° C., the time of second cleavage averages 25 minutes. The intermediate interval between this and third cleavage is unusually long; 3 observations show it to be about 75 minutes. The reason for this is unknown.

Now I wish to mention a phenomenon which challenges further careful investigation. It has been discovered that zygotes which have been rotated through 180° in position some time after having been layed, will undergo irregular cleavages. This is particularly true if the vegetal pole is placed uppermost; apparently the abnormal geotropic effect causes the pronuclei to migrate to the new "top-side." The resultant cleavages are displaced, and cut shallow, circular furrows at very off-center angles. This is reminiscent of the frog's egg upon inversion (Rugh, 1948, pp. 198-201); but the unusual effect is more easily produced in the Maryland Brook Lamprey for the egg does not rotate, and is held in place by its encasing gravel.

B. Internal Morphology

The septum of the second division cuts through the deutoplasm in the same manner as does the first. The arrangement of the yolk granules is little altered, considering the embryo as a whole.* The more plastic yolk material is centrally placed about the blastocoel which now has the shape illustrated in Fig. 50; the clefts of the two divisions are confluent. The cleft cavity is filled with a hyaline material which is probably liquid in life.

The nuclei are usually located in the animal hemisphere, about midway between pole and equator, and are close to the internal angles of the blastomeres. They are more frequently found in a kinetic state because of mitotic activity, stain poorly and the chromosomal elements cover an area about 18 μ in diameter. They are in a hyaline cytoplasm of variable size, about 55 ± 25 µ across.

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*This is not true in eggs which had been inverted after laying; in the latter there is a mixing of the deutoplasm, and the clefts between cells are greatly widened.

This clear area was observed in several cases to be bounded rather definitely by a finer layer of yolk granules, and it is characteristic of this layer of deutoplasm that many of the yolk pellets are partly stained, the colored part appearing as bands or as irregularly shaped internal structures. Under 100x the deutoplasm at times exhibits a slightly radiate arrangement of the granules, with the nucleus as a focus. This more variably stained yolk is in a concentric band which is about 140 x 200 µ in section at the nuclear level. It is surrounded in turn by the coarse, deeply staining yolk field; the latter extends to the vegetal pole except near the central clefts of the blastocoel. At the animal pole a small hyaline cap is observable, which occurs in all four blastomeres. This has disappeared after third cleavage.

5. Third Cleavage

A. External Morphology

The third cleavage, or perhaps more properly, the third generation of cleavages, takes place in a horizontal plane. These cleavage planes cut the first two septa at 90° or very nearly so. The new septa are tangent to the external surface of the embryonic sphere from 8° to 26° above the equator, in the animal hemisphere; and the result is to divide the embryo into 8 cells, 4 of which are smaller than the others. The smaller quartet (micromeres) lie around the polar axis in the upper animal hemisphere, and the larger (macromeres) in the vegetal hemisphere (Figs. 51 and 52). The macromeres contain nearly all of the grossly granular deutoplasm, and hence their descendants will divide more slowly.

The cleavages are heralded by superficial color changes in the upper animal hemisphere, which transiently appears to "blush"; that is, it becomes slightly greener about 30 minutes before the beginning of the first cleavage in a blastomere. It pales somewhat about 15 minutes later, more or less. It is inferred that this involves a relocation of the deutoplasm, for sections show that the polar cytoplasmic cap has disappeared, and some of the yolk is reoriented.

The 4 blastomeres take about 15 to 20 minutes to divide at 22° to 27° C. (4 observations). They do not cleave simultaneously but in a variable sequence, the fourth and last division starting about 6 minutes after the first. It has not been possible to determine which blastomere, if any, initiates the first of the divisions. In other words, the sequence of cleavages appears to be random. It is true also that the nuclear equatorial plates do not necessarily coincide with the horizontal plane; cleavages have been found that start vertically toward the vegetal pole, only to be deflected back to the horizontal when half completed (perhaps by the relative resistance of the coarser deutoplasm). In the 6 cases observed, the cleavage furrow starts on one side of the blastomere, and then proceeds across.

Upon completion, the horizontal furrows sink towards the center very deeply, and the effect is to lift the micromeres away from the macromeres. There is now a further separation which takes place at the animal pole where the 4 micromeres meet. The latter retract somewhat from each other at the polar axis, and appear as 4 spheroids arranged in the shape of a Greek cross. The 2 opposite micromeres are in closer contact; this requires that the longitudinal axes of the 2 pairs be curved slightly, the one at right angles over the other, the "arc" of the lower pair being inverted. During the metabolic phase after division is completed, these deep furrows become shallower and the polar cavity superficially closes; it is retained under the surface, however. The duration of the metabolic phase between the completion of the last of the third cleavages and the onset of the fourth generation averages 70 minutes (mean of 11 observations at 22° to 27° C.).

B. Internal Morphology

The overall horizontal plane of the third cleavage as viewed in embryonic midsections, is a slightly curved ellipsoid. The convexity is towards the animal pole; therefore the concavity faces the enlarging blastocoel. The vertical central radius of the convex displacement is roughly

7% of the diameter of the cleavage plane (Fig. 53). Because of the offset cleavages of the first 2 blastomeres, the outside edge of the third set of divisions is not circular but somewhat irregular. As a consequence the outside cleavage furrows in some places are farther above the equator than in others.

The micromeres are packed with the finer yolk granules. The latter average 3 x 1½ µ in size, but increase slightly in dimensions at the outside wall of the cell. There is also a thin, sticky appearing and less densely staining deutoplasmic layer facing the blastocoel, where the yolk disks may be 9 x 7 µ. Under 100x the change in density of the deutoplasm at the septa separating micromere from macromere is abrupt. There is an oblong hyaline area in the micromere, frequently near the last septum formed. A scattering of fine frothy vesicles fringes the micromeres at the time they approach or undergo division.

The macromeres have the largest yolk granules, some being 12 x 10 µ. At the external surface there is a layer of finer granules; this stratum is 25 to 40 µ wide. That part of the macromere facing the blastocoel is filled with vesicles, the larger of which may break down and open into the blastocoel. Thus "pockets" appear and the very large, sticky and lighter-staining yolk occurring here forms ragged, club-shaped strands between them. The blastocoel is now over 600 x 250 µ in section.

Measurements of a few micromere nuclei indicate cross-axes of 12 x 9 µ. There is a clearly outlined nuclear membrane, and the chromonemata can be seen easily. The nucleus is often in the hyaline cytoplasm, but it may be anywhere in the cell. In the macromeres the nucleus has the same morphology, and it lies in a small island of cytoplasm of variable size. The border between the deutoplasm and the hyaline area here is sharp.

6. Fourth Cleavage

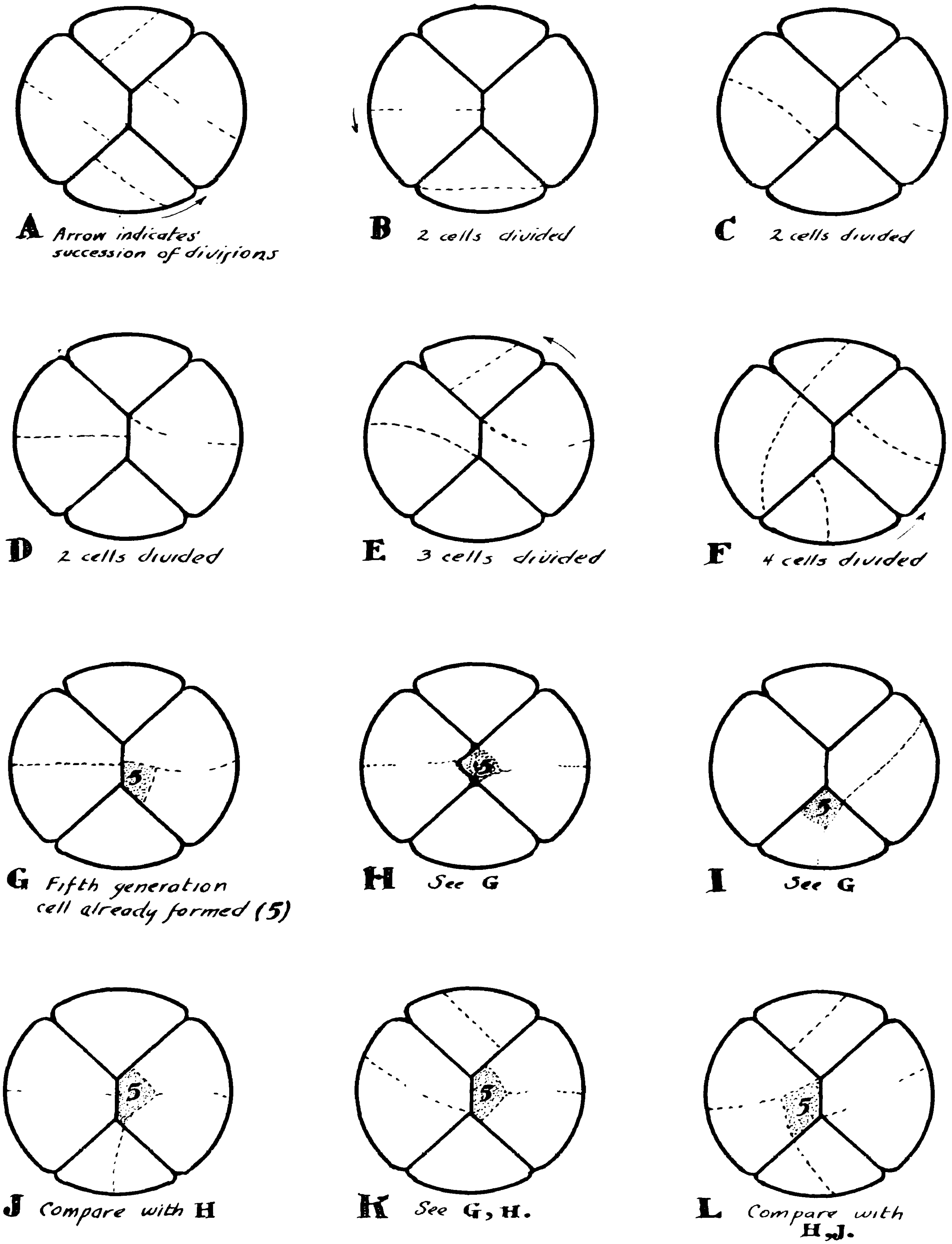
A. External Morphology

Generally, the fourth cleavage takes place in 2 steps. The micromeres divide first and then, after an interval, the macromere quartette accomplishes division. At this time, however, cleavage generations begin to overlap, because the micromeres divide much faster than the more yolky macromeres. Indeed, in one example (which appears to be exceptional) the second division in one of the quondam blastomeres had not quite been completed at the vegetal pole when the first cell of the fifth generation had been cut off at the animal pole! At the end of this generation of cleavages the embryo has the typical appearance of a blastula, for the blastocoel has the conventional relationship ascribed to that stage in a holoblastic telolecithal embryo.

Based on observations from 24 embryos, the micromeres start to divide first, and they cleave not simultaneously but in succession. The order of division is variable, but shows some tendency (6 observations) to rotate counterclockwise, as viewed looking down on the animal pole. From the beginning of the first to the completion of the fourth furrow there was an average interval of 6 minutes at 22° C. The embryo is now apparently quiet for about 16 minutes; then the macromere furrows start to become visible. The duration of this phase of cleavage is quite variable, although the furrows have formed in 8 minutes at 27° C. At 22° C. and colder temperatures the division may take over an hour, during which time the fifth generation of micromeres has proceeded far in division.

The cleavage planes of the micromeres are more or less perpendicular to the longitudinal axes of the dividing cells; but I have seen no horizontal divisions here. In the macromeres 16 observations indicate that the direction of the planes is more variable, but it is usually vertical. Nearly horizontal cleavages do occur here, however, and with some frequency. In the micromeres the division plane otherwise may be at any angle, except that the paired underlying micromeres which are separated by the dorsad meeting of the pair at right angles to them, have not been found as yet to divide in the plane of the axis.

The following diagrams illustrate some of the division patterns as viewed from the animal pole (the dotted lines are fourth generation cleavage furrows):



A Arrow indicates succession of divisions

B 2 cells divided

C 2 cells divided

D 2 cells divided

E 3 cells divided

F 4 cells divided

G Fifth generation cell already formed (5)

H See G

I See G

J Compare with H

K See G, H.

L Compare with H, J.

Diagram showing successions and variations in patterns in the fourth and fifth generations of cleavages (see text).

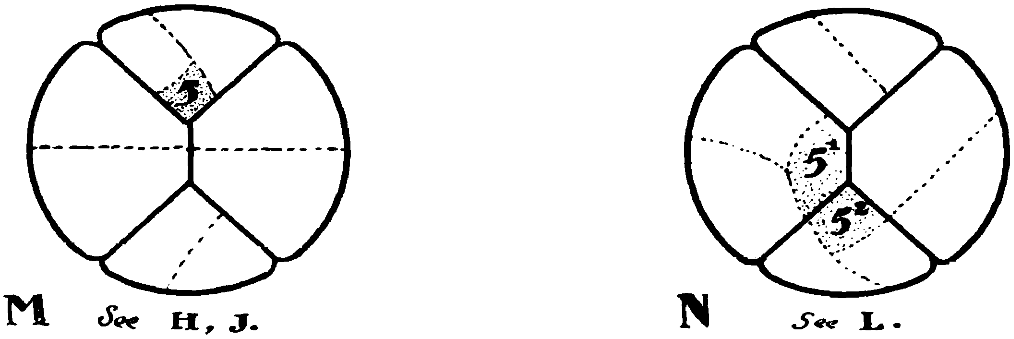


Diagram showing successions and variations in patterns in the fourth and fifth generations of cleavages (see page 77 also).

It is seen that there are 2 tendencies: (a) to divide parallel to one of the meridional cell walls already set up and (b) to start the fifth division very early by separating a cell of that generation almost exactly at the animal pole. It is concluded from this that between the third and fourth divisions the nuclei of the micromeres (and the macromeres as well) rotate about 90° in one of the cardinal directions, and sometimes will rotate to a variable degree in the other, incident to establishing cleavage divisions.

Internal Morphology

Cross-sections disclose that the micromeres are in a single layer over the blastocoel. Connections between the cells are tenuous, for the blastocoel reaches up between each cell almost to the surface of the embryo; and the roughly 80 to 200 μ wide band at the embryonic rim where cell interfaces are adjoined is interrupted by large lacunae. The micromeric layer is about 270 µ in overall thickness, although there is great variation. Under the micromeric layer, where these cells adjoin the macromeres, the latter underarch and almost meet at the polar axis, as these are shaped in their radial center planes like vaulted arches. However, between each macromere, as in the case of the micromeres, there are clefts open to the blastocoel; in sections therefore, configurations appear where the interface between micromere and macromere is very narrow.

As in the previous cleavage, the cytoplasm of the micromere shows a stratification which is somewhat parallel to the surface of the embryo. There is a deeply staining, finely granulated layer at the embryonic surface; and a spongy, more or less coarse and not-so-deeply staining deutoplasmic area facing the blastocoel. In between, except where these 2 are in contact, is an approximately lens-shaped area which is very hyaline. The cell nucleus lies in the center of the last; it has cross-axes of 11 x 9 µ and is provided with a prominent centrosphere in which there is a well-defined centriole.

The macromeres present very much the same appearance as that described under the third cleavage. The internal surface of the macromeres of a dissected egg look spongy, and under a needle behaves like friable cottage cheese.

The internal cavity is now very large, and reaches almost to the periphery of the embryo along cleavage lines;

in places it is more than 700 μ wide. As has been seen, it starts as a cleft between the first 2 blastomeres. Further development is typically that of the blastula, description of which follows.

CHAPTER V

THE BLASTULA

. General Considerations

The development of the blastula of the Maryland Brook Lamprey generally is similar to that described for L. fluviatilis (Lönnberg et al., 1924; Pietschmann, 1933). The bases for this conclusion are (a) my observations on external development in the second and third day embryo; (b) examination of slide sections, with correlation of presentations to changes in external appearance and (c) comparisons of stages of development in the Maryland Brook Lamprey with those reported for L. fluviatilis, stage for stage.

This observer divides the development of the blastula into 3 stages, not only for convenience in description, but because in each stage there is a specifically different arrangement of the micromeres and macromeres. I designate these respectively as the

i. blastula;

ii. post-blastula;

iii. intermediate stage between the typical blastula and the gastrula. I suggest the name torula for this stage (L. torulus, a little protuberance), because its chief characteristic is the development of 2 little mound-like laterodorsal outgrowths which contribute to the formation of the organizing lip of the blastopore.

These stages blend into one another successively in development, and they must not be regarded as sudden, temporarily static presentations which endure for a specific time period. They are, on the other hand, rather significant phases which are characteristic of this stage of development. I take up each of these in turn.

2. The Blastula

A. Definition

This phase is defined as that of a hollow ball of cells, whose micromeres occupy somewhat less than the upper half of the embryo (animal hemisphere), the remaining superficies being taken up by the macromeres. There is no migration of micromeres.

B. External Morphology

Beginning with the fifth generation of divisions the blastula presents a tuberculate appearance. The curved faces of the individual cells show that the smallest cells are immediately adjacent to the animal pole, and the largest to the vegetal pole. It is obvious that a metabolic gradient is operating, and the division rate seems to increase somewhat logarithmically from the vegetal to the animal pole.

In the fifth generation of divisions one of the early developments is the cleavage of a paired row of micromeres at the animal apex (3 observations). These may be formed by the gliding of cells, and the apical cells of the fifth division appear to contribute half of them (Fig. 54). Next, the hitherto uncleaved side of the original fourth generation micromere accomplishes division; but the cells resulting from this are not quite equal in size. The equatorial cells which border the macromeres are larger. This 16-or-more-celled set of micromeres coexists with a set of macromeres which may not have completed the partition of even 8 cells. At this time the embryo is at least 14 hours old (temperature 22° C.).

Subsequent divisions finally convert the animal hemisphere into a pimply dome; the increase in size of the micromeres towards the equator strongly suggests the influence of the metabolic gradient. The writer has not been able to detect any other specific cleavage pattern, however. In the later stages of the blastula the cells become stratified (see below).

. Internal Morphology

Slide sections show that the growing blastula can be regarded as exhibiting 4 levels of development:

i. The blastula consists of a single layer of cells, arranged about the blastocoel. This is typical, for example, of the 32-cell stage of development (Fig. 55).

ii. The micromeres exist in 2 layers, each layer parallel to the embryonic surface. From my observations, these layers become evident in the sixth or seventh generation of cell divisions. This stratification is the result of mitoses whose cleavage planes do not cut the external embryonic faces of the cells (Fig. 56).

iii. The micromeres exist in 3 more or less parallel layers (Fig. 57).

iv. Finally, the micromeres are found in 4 levels, and by this time the macromeres are disposed in approximately 3 layers. It is estimated that the embryo consists of over 1,000 cells at this time (Fig. 58).

At the time when the micromeres are found in 4 layers, the wall formed by them is about 200 μ in thickness; the macromere wall is 325 μ from the blastocoel to the outside. The blastocoel is ovoid in contour, and contains a hyaline, highly vesiculate interior, which gives the impression of being viscous.

At the close of this stage, the embryo has an outline very similar to that of a hen's egg. The axes of the embryo in mid-longitudinal section are of the order of 1,110 x 850 μ. The longitudinal axis appears to coincide very nearly with the polar axis, showing that there has been an elevation of the animal pole; this foreshadows the displacement of the micromeres which is about to take place.

3. The Post-blastula

A. Definition

What I term the post-blastula is the phase wherein the micromeres grow down over the vegetal hemisphere, and displace the macromeres into the future ventral side of the animal. This phase is concluded when the protuberances develop which are characteristic of the torula (cf.).

B. External Morphology

In L. fluviatilis Glaesner (1910) states that the micromeres advance posteriorly and overgrow the macromeres. The advancing edge of the micromeric sheet is raised into a ridge, which he calls a Ringwulst (i.e. a circular, ridge-like swelling). This invades the vegetal hemisphere until it forms a small elevated ring about the developing blastopore. In the Maryland Brook Lamprey on the other hand, this annular ridge is not apparent until the micromeres have almost reached the area of the blastopore. The reason for its absence is shown in slide sections; the macromeres are pushed aside or give way, and the original contour is almost completely retained. The effect of this epiboly is to cause (1) a marked flattening of the vegetal hemisphere at its pole, and finally (2) a small annular elevation through which the blastopore will be seen.

It has been stated (Pietschmann 1933) that in Lampetra this ring is eccentrically placed, and becomes somewhat ventral in a new polarity of the embryo. Investigation will be conducted to determine whether or not this applies to the Maryland Brook Lamprey as well as to L. fluviatilis.

C. Internal Morphology

During the later development of the blastula, as has been just described, the vault of the animal hemisphere is elevated considerably. This elevation continues into the present phase. I interpret slide sections here to demonstrate the following, in somewhat the order given, although more than one phase may coexist:

i. A condensation of the hitherto spongy macromeres into a more compact group of cells.

ii. A flattening of the macromeres where they face the blastocoel.

iii. A migration of some of the micromeres partly over the blastocoelic surface of the macromeres.

iv. A great expansion of the micromeric vault of the animal hemisphere. I consider that this is caused by the intussusception of once internally disposed micromeres into the more external layers.

v. A reduction to one layer of micromeres at the animal pole, and to 2 layers at about 15° from the pole.

vi. A gradual convex curving of the internal macromeres into the blastocoel, concomitant with their retraction from the original external curvature of the embryo.

vii. The epiboly of the micromeres superficially over the macromeres, the space taken by the micromeres being compensated by the inward movement of the macromeres. As a consequence there appears to be no circular ridge (Ringwulst) at this time.

viii. The progressive rounding of the macromeres into a ball.

ix. During their epiboly over the rounding mass of macromeres, the micromeres gradually develop into an organized epithelium which becomes the ectoderm. At this time the ectodermal epithelium is so reconstituted that it occupies a hollow sphere but very slightly larger than the ball of macromeres which has come to lie within it, just as a hand is drawn into a glove.

x. The blastocoel becomes an incompletely spherical cleft between the ectoderm and the macromeres (yolk cells); the latter have now become, according to the usual convention, the endoderm.

xi. Where the ectoderm develops toward the vegetal pole, it adjoins the endoderm in a very slightly raised, flange-like band. At this junction the edge of the ectoderm is elevated finally into a small, almost circular ridge. The near approximation of the circumference of this ridge closes the post-blastula period.

At the time the internal flattening of the macromeres begins, the egg is a spheroid about 850 x 950 μ in size. The migration of the micromeres on the surface of the macromeres has started (Fig. 59). At the time when the expansion of the vault of the animal hemisphere has taken place, embryonic cross-axial diameters are about 1,335 x 1,015 μ (Fig. 60).

4. The Torula

The torula is the stage of incipient gastrulation. The epithelioid ectoderm invests the spheroidal endoderm, and a thin, cleft-like blastocoel separates the 2 layers. Characteristic of this stage is the development of 2 posterodorsal paired protuberances in the vicinity of the future dorsal lip of the blastopore, and in between them there is a small elevation marking the site of the blastoporal lip (Fig. 61). A specimen of this stage fixed in alcoholic mercuric chloride solution* had a long axis (through the dorsal lip) of 1,332 μ and a horizontal width of 1,084 μ. The vertical diameter was about the same as the width.

These small hummocks appear to coalesce with the central elevation into a single ridge. This ridge, as the writer finds it, becomes a part of the contracting annular border of the ectodermal epithelium, in the center of which is the developing blastopore.

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*As used in the Feulgen technique.

CHAPTER VI

GASTRULATION

1. General Considerations

The gastrulation in the Maryland Brook Lamprey, as I have observed it, does not appear to be similar in certain respects to that described for *L. fluviatilis* by Glaesner (1910). For example, I have observed the reduction of the blastocoel to a more or less spherical cleft in the late torula, by the time that invagination begins. Therefore, the obliteration of this cavity by the act of invagination as described for *L. fluviatilis* is believed not to take place in the present species. Concurrent with this variation in development, there appears to be another. In the Maryland Brook Lamprey the epithelioidal nature of the ectoderm is quite striking, even at the beginning of invagination, and the writer is convinced that its characteristics must develop, in part at least, before this stage. The endoderm has been so designated on the basis of the same criterion. In *L. fluviatilis* however, the obliteration of the blastocoel (which is said to be accomplished during invagination) is accompanied by an inward (proximad) displacement of the macromeres. For this reason, according to this German author, the endoderm and ectoderm are not to be so termed in their characteristic relationships until after this event. Pietschmann (1935) has no different account. These differences which seem to exist in 2 species, frequently classified in the same genus, are surprising. The reader is assured that these conclusions are expressed only upon careful consideration of presentations in microscopic sections.

2. External Morphology

The projecting dorsal lip of the blastopore is the hallmark of this stage. Underneath it the blastopore itself, at first relatively wide but later a narrow slit, leads into the developing archenteron. Below this opening, the now ventral face of the embryo slopes directly into the blastoporal vestibule, since the annular ridge is now inconspicuous. This stage is achieved about or shortly after 52 hours ($20^\circ \pm 2^\circ$ C.).

The overall contour of the embryo is again shaped like a hen's egg, but it has a slightly bumpy surface. The sharper, conical apex is formed by the blastoporal lip, and the blunter, the developing anterior end. The gastrula has cross-axes of about 1-1/3 x 1-1/4 mm., as viewed dorsally. In life the color is a pale cream, and very light green ventrally just under the blastopore (Figs. 62 and 63).

3. Internal Morphology

A. Early Stage: Incipient Invagination

At the beginning of gastrulation the epithelial nature of the ectoderm is fully apparent (Fig. 64). It is a more or less columnar epithelium lying immediately under the wrinkled vitelline membrane and it invests the embryo. It is about 25 to 45 μ thick, and thinnest at the anterior end of the animal. The cells are of somewhat variable shape, ranging from columnar or cuboidal to polygonal, and are found mostly in one or two layers. The cell cytoplasm is filled with yolk granules, of which the largest are about 4 x 3 μ in size. There is a roundish nucleus (diameter ± 8 u) in a small hyaline area which is more or less centrally located in the cell. The nucleus does not stain as deeply with iron-alum hematoxylin as do the yolk granules, and it contains a more deeply staining, vesicular nucleolus. There are a few granules in the finely reticulated nucleoplasm, and occasionally an endosome of unknown nature appears in the nucleus.

The macromeric cells are packed in a sphere which occupies most of the interior of the gastrula. Since the larger macromeres were at the vegetal pole before the overgrowth of the micromeres in the post-blastula, their successors would be looked for in the vicinity of the future blastopore at this stage. This is the case; the larger endodermal cells are in the posterior part of the gastrula, although their septa are observed with difficulty. Otherwise under low magnification the cells give the impression of being arranged concentrically. Very small lacunae are infrequently found; the ball is to all intents, a solid mass (Fig. 65).

Except where the germ layers are attached in the region of the incipient blastopore, they are separated by a concentric space which on the average is about 25 μ wide. If occasional protrusions of cells (which may be artefacts) into this space are disregarded, the blastocoel appears to be empty.

In horizontal sections through the organizing lip of the blastopore, more dorsal views (Fig. 66) show a slight incurving in the posterior median edge of the lip. From the middle of this curve there is a compressed, radiate arrangement of cells growing anteriorly; it is roughly fan-shaped. These give the impression of having been rotated from without inwardly ventral to the dorsal surface, and from each side of the lip. This would cause, to be sure, the appearance of a confluence in the midline, which appears to be the case.

Serial sections cut more ventrad show a deep lateral incurving of the ectoderm. The radiate arrangement here exists both laterally and centrally; and it seems quite certain that the invagination of cells from the outside into the developing blastopore conserns the ectoderm dorsally and laterally. Most posteroventrally at this stage there is a small area of macromeres which are not covered by the epiboly of the micromeres. As a consequence, on the ventral side of the developing blastopore, the inturning cells at this time are apparently macromeric in origin.

B. Intermediate Stage (Invagination)

A longitudinal median section shows that the archenteron has developed in the midline as an extremely flattened invagination. At this stage it follows the dorsal curvature of the embryo, and is therefore curved over the endodermal mass (Fig. 67). The embryo has elongated slightly and its long axis cuts through the dorsal lip of the blastopore. It cannot be determined from available material whether or not there has been a shifting of the original embryonic axis.

The ectoderm has much the same unicellular structure as it had in the early stage just described, but in many places the epithelial cells have become more columnar in appearance. Only at the lip of the blastopore are there exterior cells of manifestly different appearance.

Under the dorsal ectoderm there is an invaginated strip of cells which lies between the archenteron and the foregoing ectoderm. This layer consists of small, polygonal cells which are spangled with yolk granules. As these cells are traced anteriorly the cellular roof which they build over the archenteron tapers to a wedge-shaped edge where it is well-advanced towards the head. These cells do not look like those of either the outer ectodermal epithelium or the heavily yolk-laden, large cells below the archenteron; for example (a) the yolk granules are intermediate in size (about 5 x 3 μ), (b) the cells are not rectangular prisms, (c) they are smaller than the macromeres and (d) they have a look of "being pushed" - i.e. the yolk granules are in transverse rows in parallel, and the long axes of the cells lie horizontally across the layer. These cells also extend laterally under the ectoderm, and consequently form a somewhat shield-shaped lamina which converges toward the top and sides of the blastopore. I have not been able to distinguish any stratification within this layer; from its position, undoubtedly it includes the anlage of the mesoderm.

A median longitudinal section taken at the curvature of the dorsal lip of the blastopore shows that there is a small group of cells which have a triangular shape. The apices of these cells are directed towards the blastocoelic cleft which stops at this point. The demarcation between these and the cells of the ectodermal epithelium is quite obvious; within a progression of 1 or 2 cells, the histological appearance has changed (Fig. 68). The same observation holds for the translation of form to that of the cells of the archenteric roof.

At the ventral lip of the blastopore the forces of invagination appear to influence strongly the movement of the posteriorly situated, large yolk cells. Mesially, the endoderm containing the largest yolk granules comes to lie in the floor of the primitive gut, and its yolk cells are carried apparently by convection also to the center of the endodermal mass. A somewhat stellate cleft sometimes appears in the middle of this slowly turning germ layer; but the cleft seems to be adventitious and later it disappears. At this stage the ventral ectoderm does not appear to sweep into the blastopore; it tapers to a smooth edge where it still coincides with the outer ovoidal contour of the embryo.

One result of the development of the primitive gut is to establish new relationships between the large, heavily yolk-laden endodermal cells and those invaginated dorsally and laterally. There seems to be an anterior and lateral upward displacement of the erstwhile macromeres about the periphery. This gives the impression that the cavity of the archenteron is projecting forward into the endodermal mass. In the meantime the advancing frontier of the new layer of cells (inturned and lying between the dorsal ectoderm and the archenteron) infiltrates progressively between the ectodermal epithelium and the coarsely granuled endodermal cells. The first narrow boundaries between these 2 cell groups are uneven and ragged, and sections show scattered, heavily yolked cells pegged in between those of the new layer. Therefore, the endodermal cells which are to pave the ceiling of the primitive gut are, at this time, in an illy defined state.

. Late Stage (Pro-neurula)

Slide sections of a stage of about 66 ± 4 hours (at 20° ± 2° C.) show that the 3 germ layers can be identified with certainty. The blastoporal vestibule is still manifest as a relatively large, cone-shaped recess in the posterior face of the embryo; the slit-like pore so characteristic of the neurula has not yet developed. The embryo is still ovoidal, and it has approximately the same axial dimensions as in the early stage (*q. v.*).

Significant developments in the ectoderm are now taking place. Laterally and ventrally the ectodermal epithelium has become thinner; it is about 18 to 20 µ thick. A much thicker longitudinal strip of ectoderm extends from the dorsal lip of the blastopore far anterad; it curves for about 150° around the dorsal embryonic outline, and already shows a slight ridge-like elevation anteriorly. Here it is about 200 µ thick and 250 µ wide. This is the medullary plate which is ancestral to the nervous system.

The medullary cells appear to be the smallest in the embryo at this stage. They are covered dorsally by an epithelioid layer which is continuous with the rest of the medullary cells and with the outer ectodermal epithelium; and the cells lining the plate ventrally likewise are somewhat stratified. The remainder of the cells are not obviously in any specialized arrangement, but they are very compactly associated and are filled with only the finer yolk granules. In longitudinal sections the thickness of the plate diminishes somewhat posterad; near the dorsal lip where the medullary cells give way to the more totipotent cells of that region, it is less than 45 μ thick.

The mesoderm can be traced to a germinally fused area at the dorsal and lateral edges of the blastoporal orifice. Study of progressive sections convinces me that most of the mesoderm, at least, is not endodermal in origin. To be sure there are, during earlier stages of gastrulation, scattered cells which are found in close association with the archenteric surface of the intermediate layer, and which from their size, high deutoplasmic content and coarseness of yolk granules are most evidently endodermal. The mesoderm has grown anterodorsally and somewhat laterally between the endoderm and the ectoderm, although it has not yet met in the ventral midline. It does not exhibit the coelomic split and other reshaping which will characterize the myomeres and the somatopleure and splanchnopleure.

A study of the development of the notochord is reserved for incorporation in future studies of organogeny. The development of the germ layers and the advent of differentiation of the medullary plate closes gastrulation.

CHAPTER VII

EXTERNAL MORPHOLOGY OF DEVELOPMENT: NEURULATION TO HATCHING

. General

In the earlier stages of the present research, developing embryos were observed and then immediately fixed, embedded and sectioned, as studies of slide sections were scheduled first. As a consequence, no pictures of entire mounts were obtained for illustration until April, 1952, during part of which time this paper was being prepared. At the time this paragraph is written (April 20, 1952) the writer has secured embryos in every stage from that of the fertilized zygote to the 18th day (spawning was consummated in a laboratory aquarium on April 2). The temperature of the water in the aquarium fluctuated between 18° and 22° C., with rare extremes of 16° to 23° C (the aquarium is in a screened open window in a room which has not been heated during this time). Therefore, a brief description is now included of some of the later stages as shown by whole mounts.

For convenience, the sections which follow are titled:

i. Neurula
ii. Head extension
iii. The larva at hatching

2. Neurula

Stages showing neurulation were taken from the aquarium on the 4th day after spawning. Hence the embryos described here were from about 70 to 90 hours old. The embryo elongates at neurulation to about 1.6 mm. in length and it is 1.25 mm. wide as viewed from above. Postero-ventrally the embryo has the characteristic light-green color which is associated with the original vegetal hemisphere. Elsewhere the animal is cream-colored to white, the last color appearing dorsally.

The development of a dorsal, somewhat ribbon-shaped thickening of ectoderm indicates that the anlage of the nervous system is being established (see also under Gastrulation). The thickened plate grows anteriorly and at the head end begins to lift away from the curvature of the anterior face of the embryo. This creates the appearance of a small, crescent-shaped ridge quite anterodorsally; the horns of the ridge point posteriorly so that the curve of the ridge is athwart the central longitudinal axis of the embryo. The points of the crescent extend as low folds which run posteriorly towards the dorsal lip of the blastopore. They are almost parallel; they converge towards the posterior point (Figs. 69 and 70). Between the folds there develops a central trough which deepens to a furrow, more deeply at first in the mid-dorsal point, and thence simultaneously anteriorly and posteriorly. These are the medullary folds which become neural ridges, and the furrow is the neural

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groove. In cross-section, the plate becomes strongly curved, and the neural ridges rise higher and turn over dorsally towards the midline until they meet (Figs. 71 and 72). The nerve cord so formed sinks below the over-closing epithelium and leaves a high, peaked ridge extending in the dorsal midline from the most anterior point of the embryo to a recurved posteroventral termination.

During this process, the blastopore narrows to an inconspicuous transverse slit (Fig. 73).

Embryos collected 100 to 120 hours after spawning show that the nerve cord has been surrounded by the expanding epimeres, and the dorsal ridge now has a distinct shoulder, particularly in the future head region. The blastopore is now identified with the anal region, and is minute in size.

3. Head Extension

The head extension stage has been found to exist from about the 165th to the 250th hour of age (20° ± 2° C.). The embryos begin to hatch in the laboratory beginning with the 11th day.

During the head extension stage organogeny is rapidly taking place. The metabolic gradient which had manifested itself so strikingly in the third generation of cleavages again indicates that the anterior region is the zone of the most rapid growth. The rapidly forming head projects itself forward and downward, and is appressed against the ventral ectoderm. Its curvature is sharp and forms a cervical flexure, the most anterior part of which is used in measurements.

At the beginning there is an anterior knob-like projection which is continuous with the highly elevated dorsal ridge in which the developing brain and spinal cord is found. This projecting knob is shown by sections to contain the forebrain. Laterally and slightly posteroventrally, there is an inconspicuous optic cup. At this time the cervical flexure - "rump" length is 1.6 mm. and the greatest width from the dorsal aspect is 1.2 mm. (Fig. 74). The head extension itself is about 450 mm. in height from the point of the sharp internal angle where it bends over the ventral wall, to the top of the dorsal ectodermal wall over the nervous system.

As the head end elongates it continues its reflex curvature, and the globular posteroventral part of the animal correspondingly shrinks in size. In this transition process it looks now like a comma when viewed from the side. At first the central axis of the head and that of the posterior part of the body lie in the same dorsoventral plane; but as the animal elongates there may be a slight torsion and the head may be twisted so that, for example, one side rests against the ventral curvature, but on the complementary ventral side of the animal. This posture has been seen, in a number of cases, but there is no indication that this torsion is part of a basic growth pattern. Also, I have seen embryos where the elongating head remained in the longitudinal dorsoventral plane of the embryo except at the end of the "U"-stage discussed below.

The abdominal curvature is often reshaped to accomodate the growing head. That is, the anterior, upswinging curvature of the ventral surface over which the head projects, often develops a sort of recess into which the head fits (Fig. 75).

The middle period of head extension is considered to have been achieved when the embryo assumes the form of the letter U; that is, the recurved head extension has about the same length as the rest of the body. Internally at this time there are 3 spiracular indentations in the pharynx on each side, but they do not open to the outside. Externally the spiracular area is characterized by a series of low, convex placodelike formations, between which eventually the external branchial clefts will form (Fig. 76). This stage is achieved in the early part of the 10th day under laboratory conditions.

Beginning with the 11th day, the embryo in the laboratory aquarium begins to straighten out; and as it does so, the head ruptures the vitelline membrane. It soon ruptures the gelatinous covering as well. In straightening out, the embryo takes a form which Hatta called "pistolformig" --- i. e., shaped like a pistol. The handle of the pistol would be the still somewhat curved, yolk-lined abdomen.

4. The Embryo at Hatching

The embryo at hatching is roughly 3.4 mm. long, along the chord of the arc formed by the curvature of the body. The yolk is concentrated around the alimentary tract, which bulges on account of it. The integument is a thin, transparent epithelium through which in the living animal the beating heart may be clearly seen. The spiracular clefts are not patent, but the future oral vestibule is deeply inset. The caudal fin has started to form a small crest and most of the myotomes can be seen easily through the integument. They diminish in size posterad and caudally cannot be counted with certainty.

The reader is referred to Figs. 77 and those following, for the general appearance of the larva at this time and at later intervals. As I conclude this there is an 18-day old larva swimming in a small bowl in front of me. At intervals it seeks to hide under some rice grains placed in the water. All of its spiracles are functioning and the larval hood has already formed. A lateral black dot on each side in the region of the brain identifies the eye, and the pulsating heart winks redly in the light.

CHAPTER VIII

DISCUSSION AND SUMMARY

1. Discussion

One of the reasons why the present investigation has been so challenging is that relatively little has been published on the embryology of American brook lampreys. Another is that although eastern American museums have specimens of *L. aepyptera* in their cabinets, the Maryland Brook Lamprey has remained one of the more obscure forms. This is because it has been the subject of infrequent study and its classification is apparently still in need of further investigation. Again (and this is by no means last or least), the present investigation has convinced the writer that some currently accepted accounts of embryonic development in such lampreys as *L. fluviatilis* need to be rechecked in the light of present-day knowledge of embryonic development. (Lönnberg *et al*., 1924, and Pietschmann, 1933, with particular reference to their pertinent bibliography). The literature relating to *L. aepyptera* has been concerned primarily with taxonomic criteria, distribution and adult behavior.

Okkelberg (1918) issued an extensive and detailed paper on the early development of the reproductive tract in *Entosphenus lamottenii* (= *E. wilderi*, *E. appendix*, *L. wilderi*, *L. lamottenii*); and Vladykov (1950) has published recently some valuable information on the larval form of *L. aepyptera*. It remains true, however, that descriptions of embryonic development of only a few species of lampreys constitute most of the literature. These species are, in the main, *Petromyzon marinus*, *Lampetra fluviatilis*, *L. planeri* and *L. mitsukurii* (= *L. reissneri*). See the works of Ballowitz (1905); Bataillon (1904); Bühler (1902); Eycleshymer (1895); Glaesner (1910, 1910a); Hatta (1892, 1907, 1908, 1922); Keiser (1914); Lubosch (1904); Scott (1882) and Selys-Longchamps (1910). Recent reviews have been quoted.

The development of the gametes in the Maryland Brook Lamprey appears to be very like that for brook lampreys in general. However, it is different in certain particulars, for example, from that of *L. fluviatilis* as described by Lubosch. He was of the opinion that he could distinguish oögonia in 6 mm. larvae, and described their nuclear structure in terms of concepts prevailing in 1904. One of his most interesting statements as quoted by Lönnberg *et al.* (1924) is that the germinal vesicle prior to meiosis diminished in volume by extruding its nucleoplasm, while the *nucleolus* transfers its affinity for nuclear dyes to the chromosomes *arising from it*:

> ....dass es unter Abgabe des Karyoplasma an Volumen sich verringert, während der Nucleolus .... die Affinität zu Kernfarbstoffe auf mehrere aus ihm entstehende Chromosomen überträgt.

This work is the basis for descriptions of this phase of development in the German encyclopaedic treatises.

The sequence of configurations presented on slides of the maturation of the male sex cell closely parallels those of vertebrates in general. The structure of the spermatozoon is so like that described for other representatives of the genus Lampetra that it encourages a belief that the Maryland Brook Lamprey is indeed to be placed in that genus.

Another variation in the sequence of development which may be noted here is that from the late blastula to gastrulation. Glaesner's (1910) drawings of early gastrulation in L. fluviatilis are not at all similar to presentations on my slides. Rather, the large, bladder-like structure which he has drawn reminds me of certain aspects of sections of the post-blastula (q. v.) particularly if the latter has somewhat collapsed in sectioning and embedding.

Pietschmann (1933) reports a 3-year life cycle for the European brook lamprey; and in other respects the life history of the Maryland form is a close reflection of that of other non-parasitic, rivuline species.

2. Summary

The Maryland Brook Lamprey, provisionally identified as Lampetra aepyptera, has been reported from tributaries of the Patuxent River in Maryland since 1888. Specimens used in the present research were taken from Crow and Bear Branches near Laurel, Md., during the period 1950-1952; it is a typical non-parasitic brook lamprey. During this time no other species of lamprey has been found to coexist in the same Branches.

This lamprey is small (104 mm. average length). The adult has the characteristics of the genus Lampetra; it is slate brown to deep raw-umber dorsally and has a glistening, cream-colored abdomen and spiracular area. There are 2 dorsal fins dorsoposteriorly, the more posterior the larger; and terminally the animal has a diamond-shaped caudal fin. In the adults the intestinal tract degenerates to a useless strand. Its dentition, fins and myotome count indicate a presumptive classification as Lampetra aepyptera.

The life cycle is considered to be 3 years. Spawning takes place from about April 10 to May 10 in concave nests in sandy gravel where there is clean, shallow brook water. The eggs are fertilized externally after a characteristic "wrestling" of the mating pair; the zygote develops a viscid, gelatinous coat which causes it to adhere to the substrate and to be covered with sand grains. The parents soon die after spawning.

Studies of the growth rate indicate the following average lengths of the larva at the end of each year: first year, 34 mm.; second year, 87 mm; third year (before external metamorphosis) 110 mm. The ammocoete is uniformly brown, and has a larval hood, reduced fins, and undeveloped eyes.

Gametes are relatively undifferentiated until the beginning of the second year. The typical oögonium is organized in the 37 mm. larva by the contribution of deutoplasmic material to 1 cell in the follicle by most, if not all of the others. Thenceforth the egg grows until it achieves a diameter of 900 μ. Meiosis I in the egg occurs in October of the third year at the 39th parallel and Meiosis II takes place at fertilization, the secondary oöcyte metaphase being static just before spawning.

The first maturation division in the primary spermatocyte occurs in the latter part of October and the first part of November in the third year, and Meiosis II then follows in the next February. The male sex cell completes its transformation to the mature spermatozoon in March. The latter has a lancet-shaped head about 12 μ long, a connecting piece, and a very long, fine tail filament of indeterminate length (over 200 μ). There is also a very fine head filament at least 13 μ in length projecting anteriorly from the head.

At spawning the ovum has a relatively thick vitelline membrane in which there is no micropyle. The egg is quite yolky and most of the clear cytoplasm is at the animal pole. Extrusion of the second polar body indicates the formation of the haploid female pronucleus; the mating pronuclei meet and organize the first cleavage metaphase in the animal hemisphere. This preparatory period takes at least 6 hours under laboratory conditions.

Cleavages are holoblastic and unequal.

First cleavage is vertical and starts at the animal pole. It takes about 2 hours (all time observations were made under laboratory conditions, temperatures 18° to 27° C.). The 2 blastomeres endure for about 35 to 40 minutes.

Second cleavage is likewise meridional and at right angles to the first; and the division planes in each blastomere are usually slightly offset. The division is accomplished in about 25 minutes and the following 4-cell stage lasts about 75 minutes.

Third cleavage is horizontal or very nearly so, but varies in level from 8° to 26° above the equator. It forms in about 15 to 20 minutes and divides the egg into 4 smaller micromeres in the animal hemisphere and 4 yolky macromeres in the vegetal hemisphere. The ensuing metabolic phase lasts about 70 minutes.

In the fourth cleavage the micromeres divide first, thus indicating the presence of a metabolic gradient. They cleave mostly in succession, and all have divided in about 6 minutes. After about 16 minutes cleavage furrows appear in the macromeres. These last cleavages overlap the advent of the fifth generation of cleavages in the micromeres. These divisions are more or less perpendicular to the surface.

During the fifth cleavage the embryo is obviously a blastula. It is a hollow ball with rapidly dividing micromeres located initially in a single layer in the animal hemisphere, and has a vegetal layer of more slowly dividing macromeres. The embryo still has approximately the same dimensions as the fertilized zygote. The ever smaller but rapidly dividing micromeres proliferate up to 4 concentric layers of cells in the vault of the animal hemisphere, the more slowly dividing macromeres up to 3 layers.

The cell displacement which now occurs in the blastula converts it into the post-blastula. The micromeres migrate and thin out into a larger, 1- to 3-cell layered dome-like structure, and the macromeres first flatten out and then reform into a ball in the floor of the vegetal hemisphere. The micromeres then grow or extend over the macromeres, during which time they become epithelioid, and at the end of this stage the inner macromeres fit closely into the ovoidal micromeric capsule. The micromeric and macromeric layers are conjoined in a circle near the pole of the vegetal hemisphere; in the center of this circle there will develop a blastopore. The micromeres and macromeres are separated elsewhere by the spheroidal cleft of the blastocoel.

In the next (torula) stage there develop 2 postero-dorsal protuberances in the vicinity of the future dorsal lip of the blastopore. These fuse in the midline. The blastopore now shows as a conical depression incident to the coming gastrulation.

The gastrula is found on the third day. The archenteron is accomplished by epiboly and involution of ectoderm and endoderm through the blastopore. There is a well-defined organizing gradient in the now posteriorly projecting dorsal blastoporal lip. The archenteron is deflected dorsally, and is lined with endoderm. The infolding layer of cells between the archenteron and the dorsal ectoderm furnishes (among other things) the anlage of the mesoderm. The ectoderm thickens in the dorsal midline to form the medullary plate. Embryonic cross-axes at this stage are about 1-1/3 x 1-1/4 mm.

The formation of the medullary plate from contiguous ectoderm signals the start of organogeny. Neurulation is conspicuous on the fourth day, and is characterized by the formation of longitudinal ridges which are broadly connected at the head end by an anteriorly convex, crescent-shaped elevation. These ridges approximate each other dorsomesially and fuse; and the resulting spinal cord and brain formed by them sink below the overgrowing ectoderm. The embryo is now about 1-3/5 mm. long.

The head then projects out from the rest of the ball-like embryo, and grows proportionately more rapidly in the head extension stage. This development occurs from about the 165th to the 250th hour. The embryo is whitish, "comma"-shaped from side view, and shows its knoblike developing forebrain and depressions for future spiracular openings. As it grows, the embryo becomes U-shaped and then straightens out.

Embryos hatch beginning on the 11th day in the laboratory. They are then slightly curved, somewhat clavate animals of 3.6 to 4 mm. in length. The yolk is now concentrated around the alimentary tract, which still bulges. The mouth and spiracles are still closed, but the musculature is capable of some movement, and the heart is functioning.

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DEVELOP THE GAMETES AND EARLY EMBRY TH PLATE
ARYLAN LAMPREY (*LAMPETRA AEPYPTERA*, 1

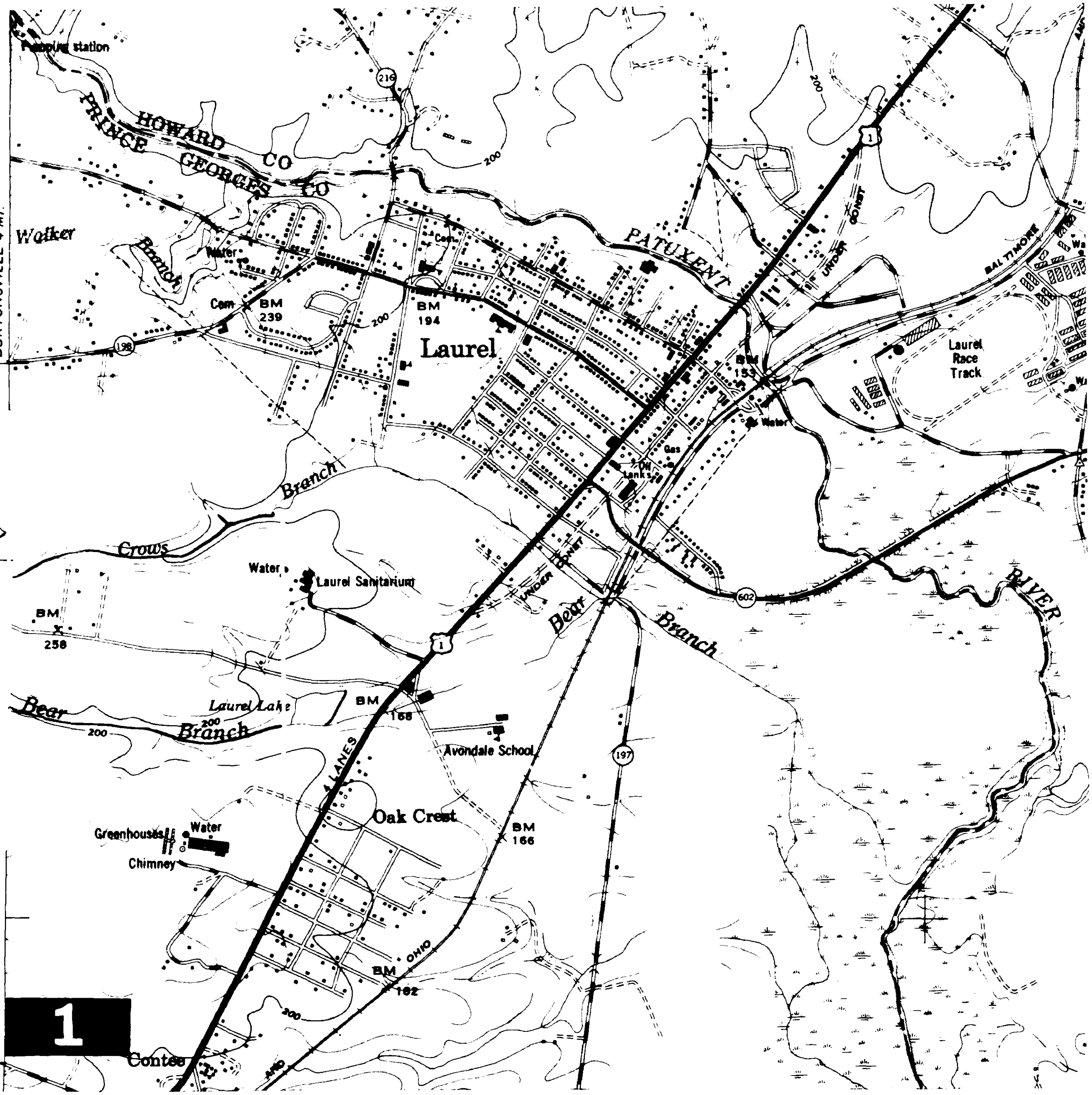

7.1 MI. TO U.S. 29
BURTONSVILLE 4 MI.
Pumping station
216
HOWARD CO
PRINCE GEORGES CO
200
1
PATUXENT
UNDER CONST
BALTIMORE
Walker
Branch
Water
Cem
BM 239
BM 194
Laurel
198
BM 153
Laurel Race Track
Water
Gas
Oil tanks
Branch
Crows
Water
Laurel Sanitarium
BM 258
602
RIVER
Bear
Branch
UNDER CONST
Laurel Lake
Bear
Branch
200
BM 168
Avondale School
197
4 LANES
Oak Crest
BM 166
Greenhouses
Water
Chimney
OHIO
BM 162
1.9 MI. TO MARYLAND 198
1
Contee
AND

PLATE II

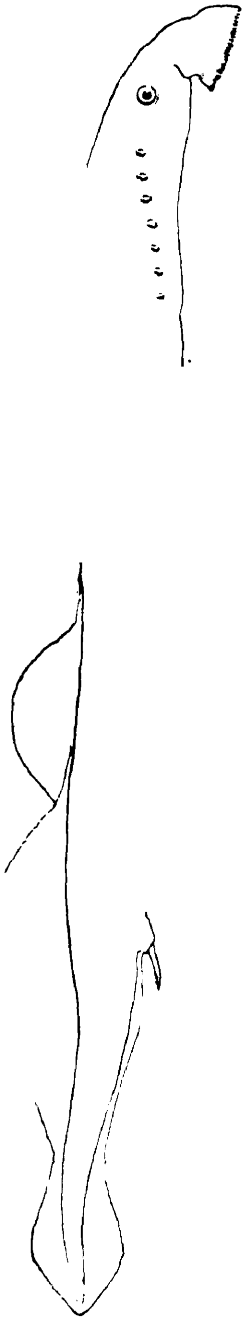


FIG. 2

Adult Male
1-2/3X

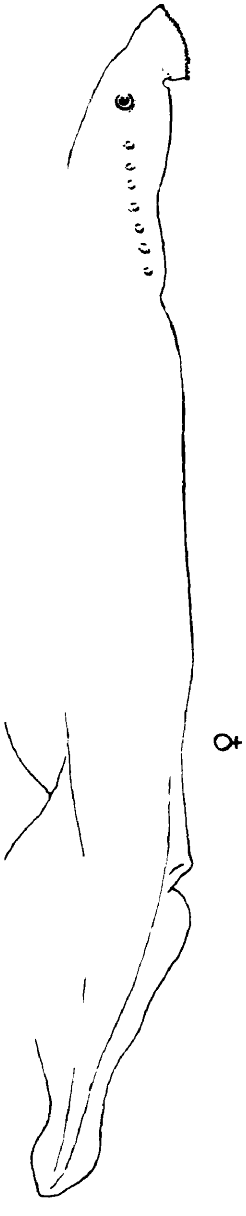


FIG. 3

Adult Female
1-2/3X

Text reference: page 5

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE III

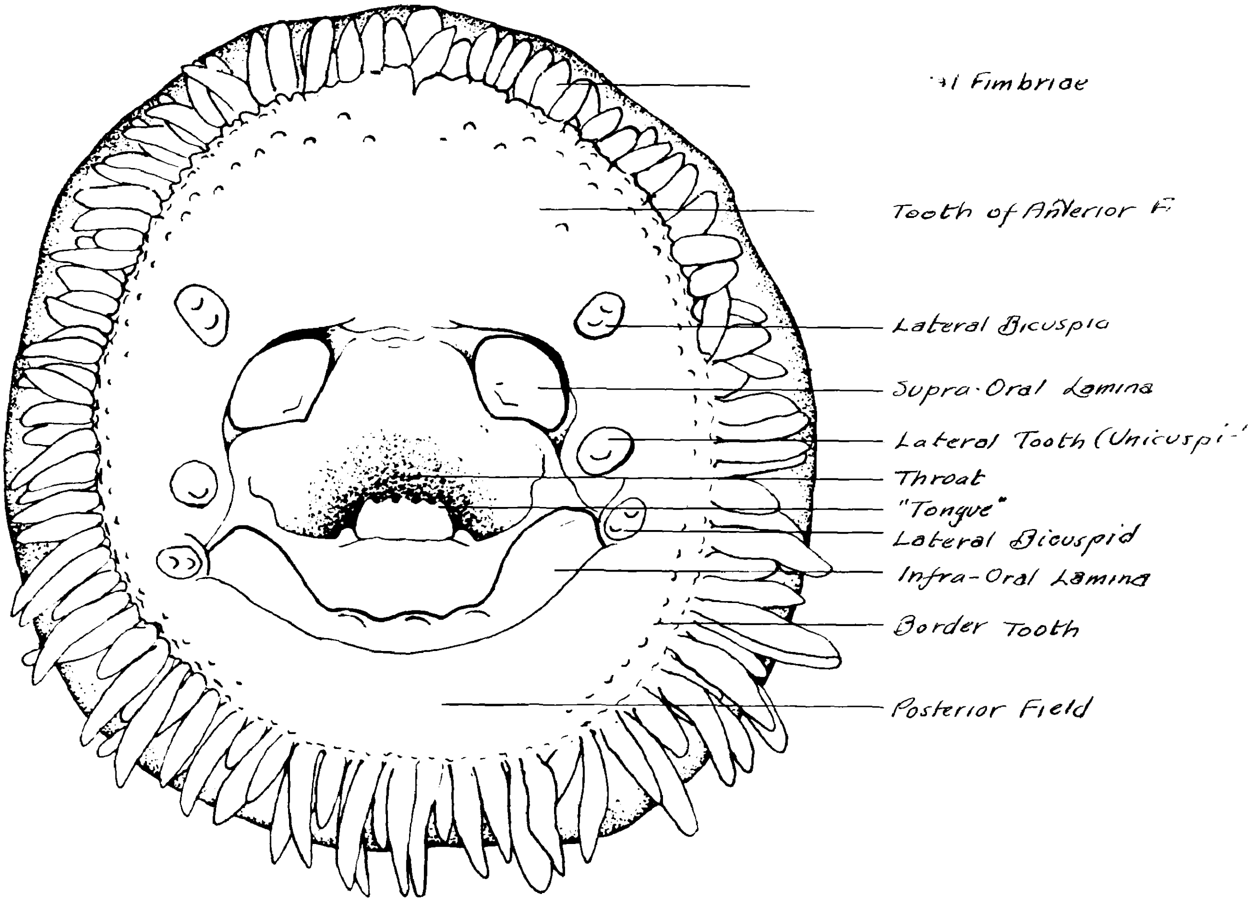

·al Fimbriae
Tooth of Anterior F
Lateral Bicuspid
Supra-Oral Lamina
Lateral Tooth (Unicuspi
Throat
"Tongue"
Lateral Bicuspid
Infra-Oral Lamina
Border Tooth
Posterior Field

FIG. 4a. Oral disk, Maryland Brook Lamprey

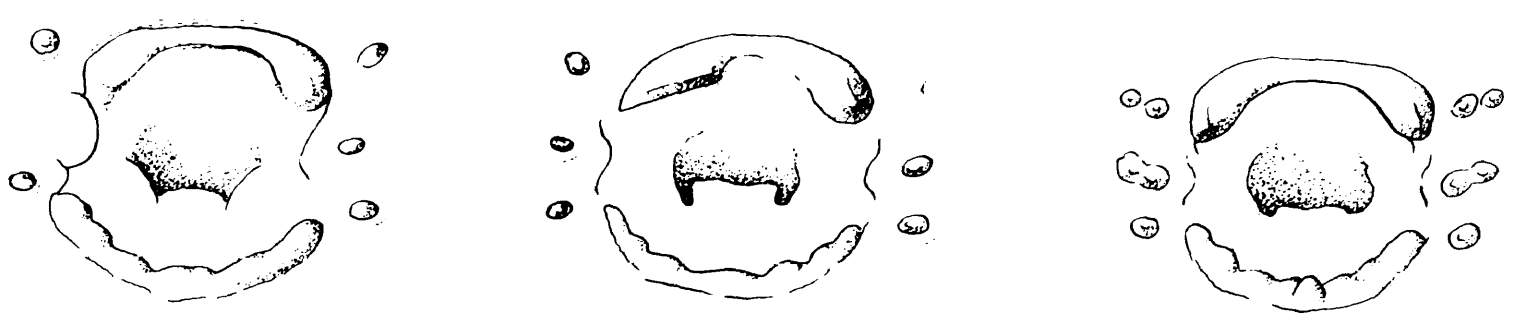


FIG.

FIG. 4c.

Variation in teeth patterns

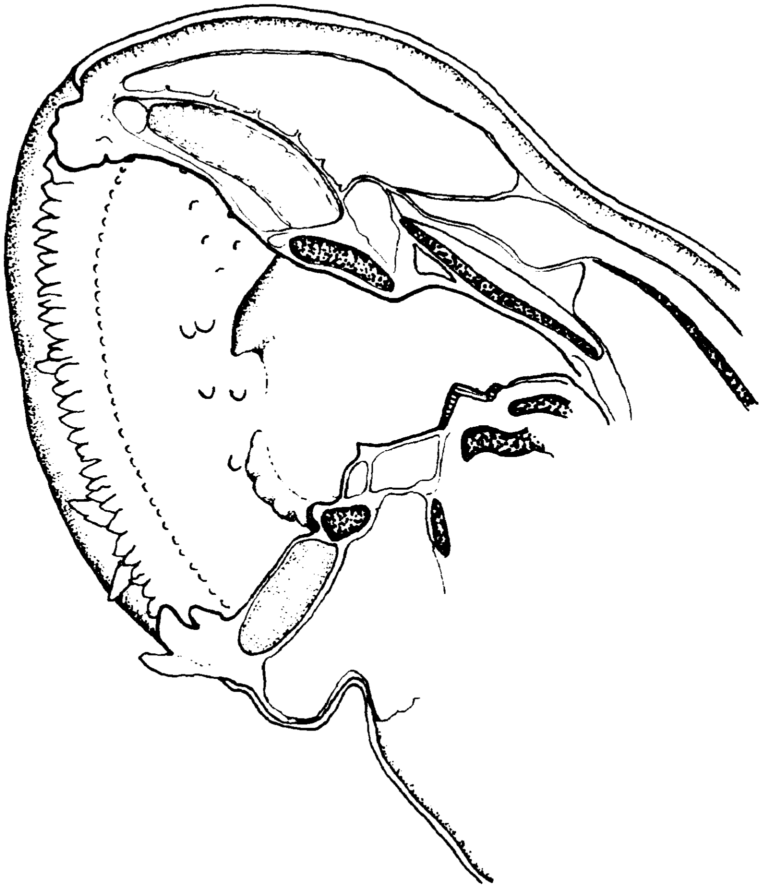


FIG. 4e. Median Longitudinal Section

Through Buccal Funnel

About 15X
(See also FIG. 4a

Text reference: page 1

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE V

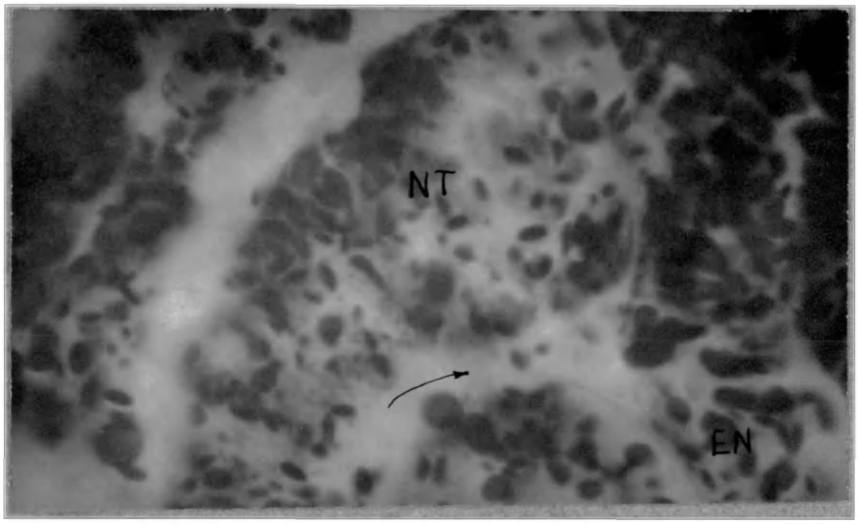


FIG. 5.

Possible undifferentiated sex cells.

From 1,455X

NT: Probable notochordal anlage
EN: Endoderm

Text reference: p. 33

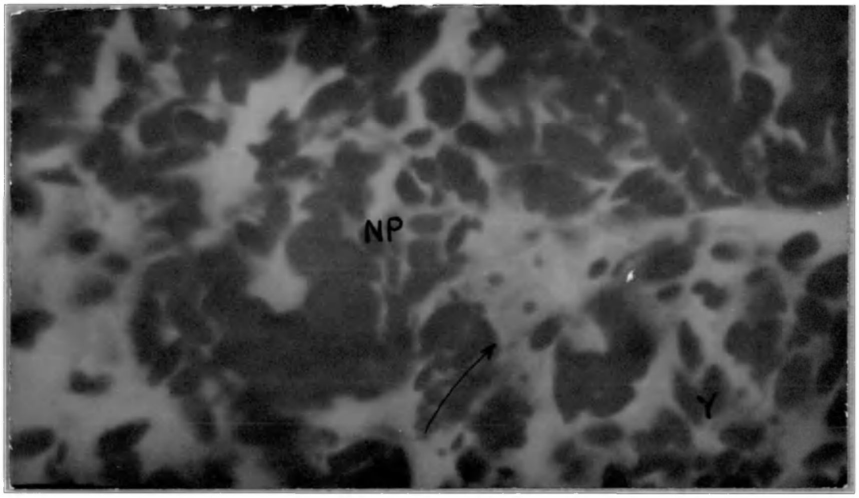


FIG. 6.

Undifferentiated sex cells, 2.50 mm. larva.

From 1,455X

NP: Nephrotomal area
Y: Yolk

Text reference: p. 34

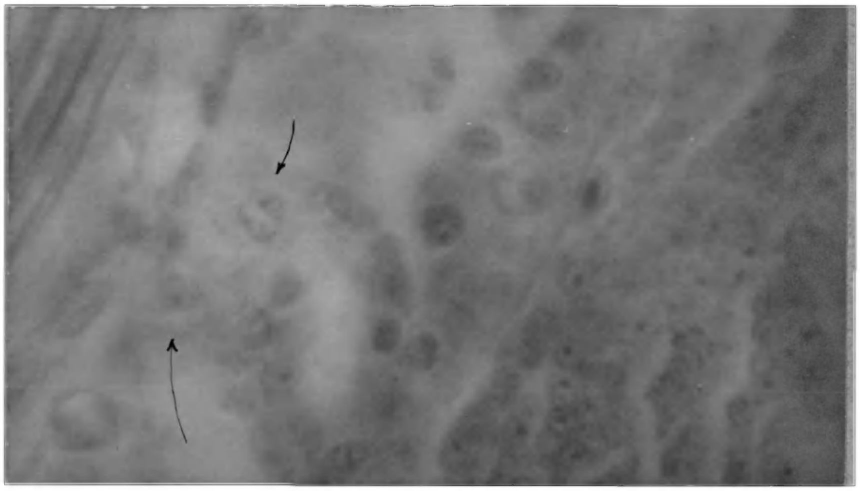


FIG. 7.

Undifferentiated sex cells, 5.95 mm. larva.

From 1,455X

IN: Intestine
MS: Mesonephric tissue

Text reference: p. 34

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE VI

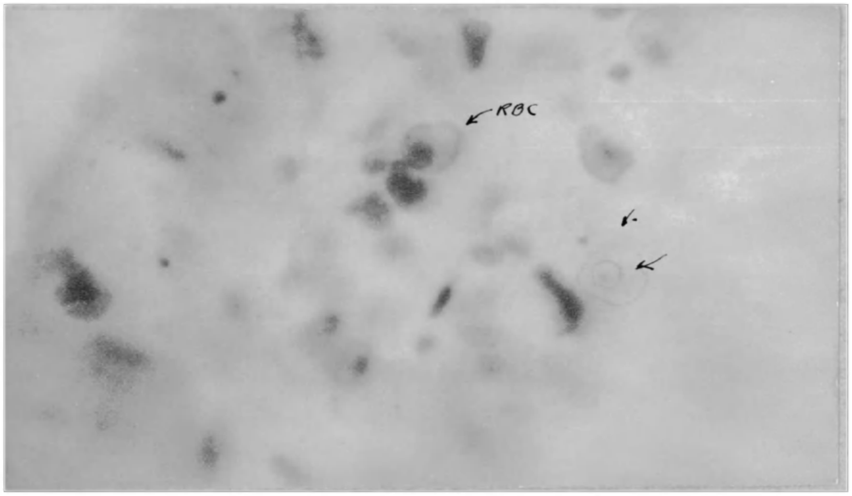


FIG. 8.

Undifferentiated sex cells, 11.5 mm. larva.

From 970X

RBC: Red blood corpuscle

Text reference: p. 35

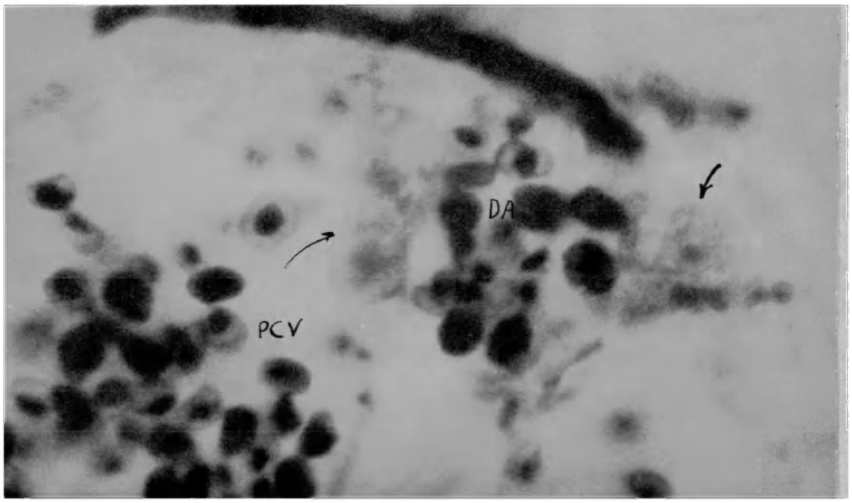


FIG. 9.

Undifferentiated sex cells, 13.75 mm. larva.

From 970X

DA: Dorsal aorta
PCV: Post-cardinal vein

Text reference: p. 36

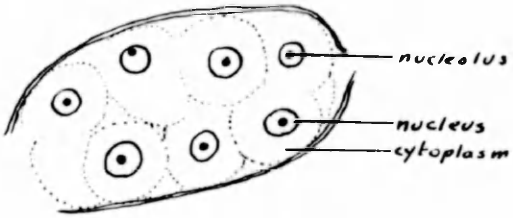


FIG. 10.

Undifferentiated sex cells, 17 mm. larva.

From 970X

Text reference: p. 37

PLATE VII

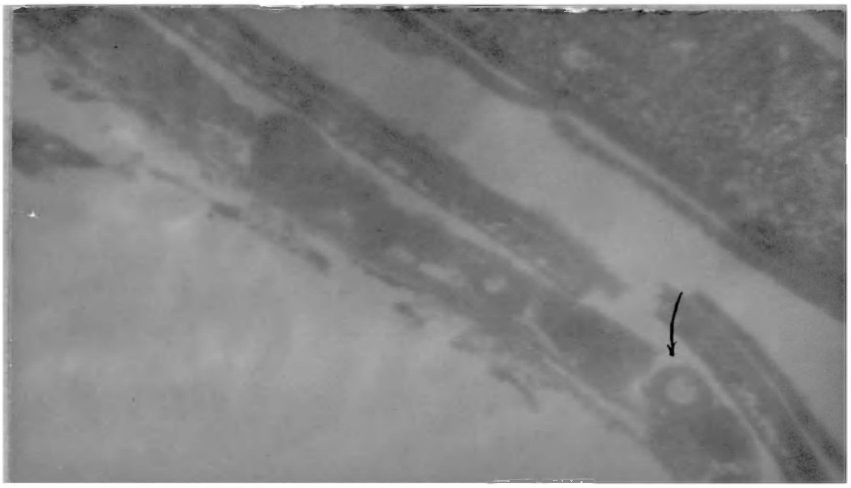


FIG. 11.

Differentiating oögonia, 37 mm. larva.

From 100X

Text reference: p. 37

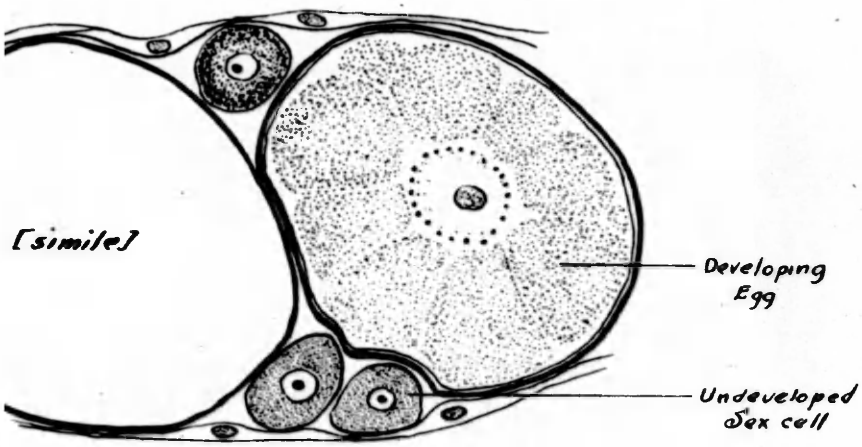

[simile]
Developing Egg
Undeveloped Sex cell

FIG. 12.

Differentiating oögonia, showing undeveloped sex cells

About 650X

Text reference: p. 38

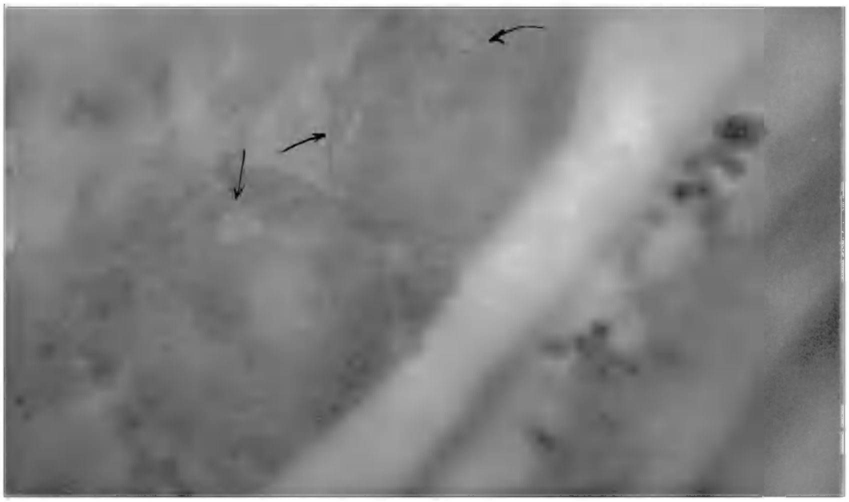


FIG. 13.

Differentiating oögonia, showing nuclear degeneration of accessory cells

From 440X

Text reference: p. 39

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE VIII

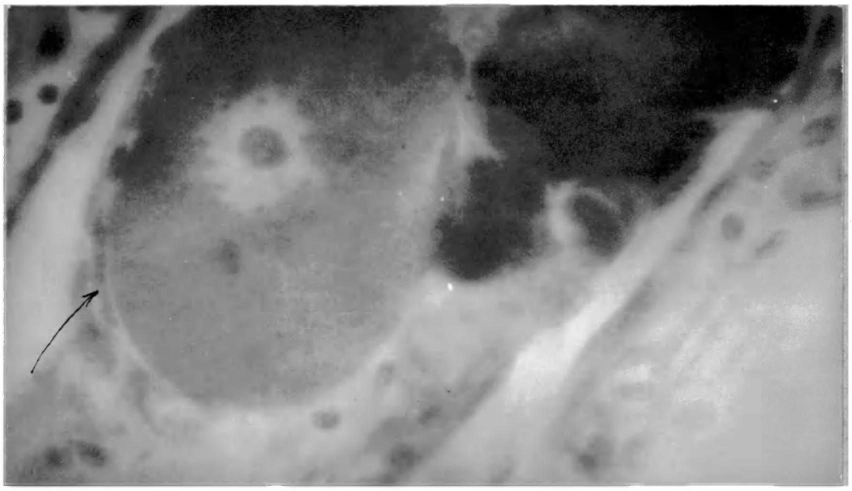


FIG. 14.

Differentiating oögonia, 37 mm. larva. Accessory nuclei are disappearing.

From 440X times 2

Text reference: p. 39

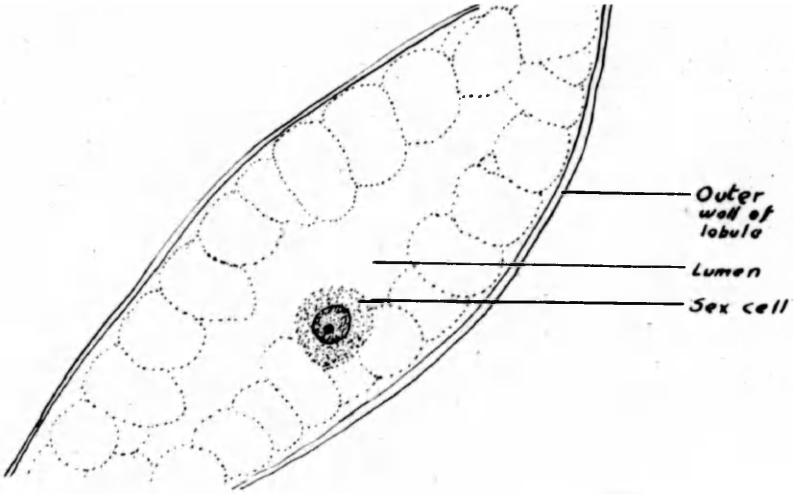


FIG. 15.

Differentiating spermatogonia, 46 mm. larva.

From 1,455X

Text reference, p. 39

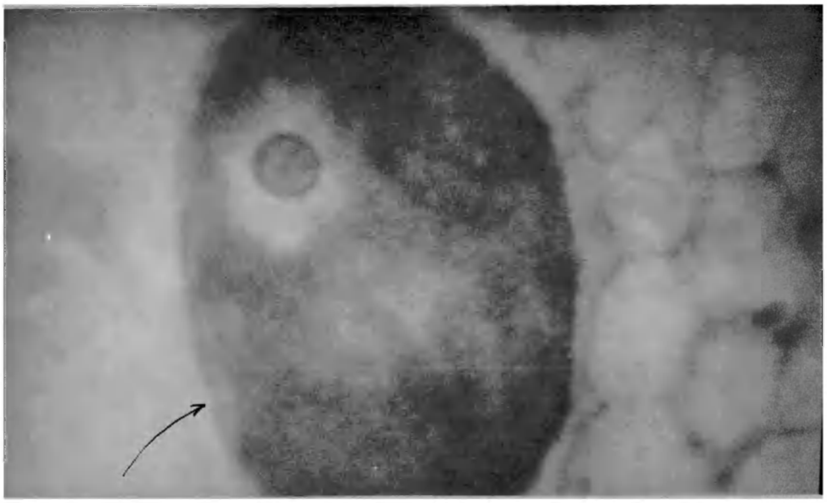


FIG. 16.

Oögonium, 76-1/2 mm. larva.

From 440X times 2

Text reference: p. 40

PLATE IX

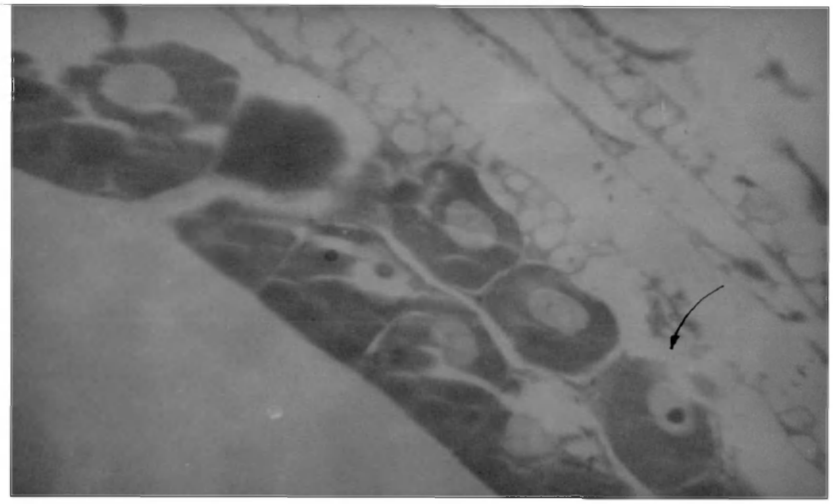


FIG. 17.

Oögonium, 101 mm. larva.

From 100X times 1.4

Text reference: p. 40

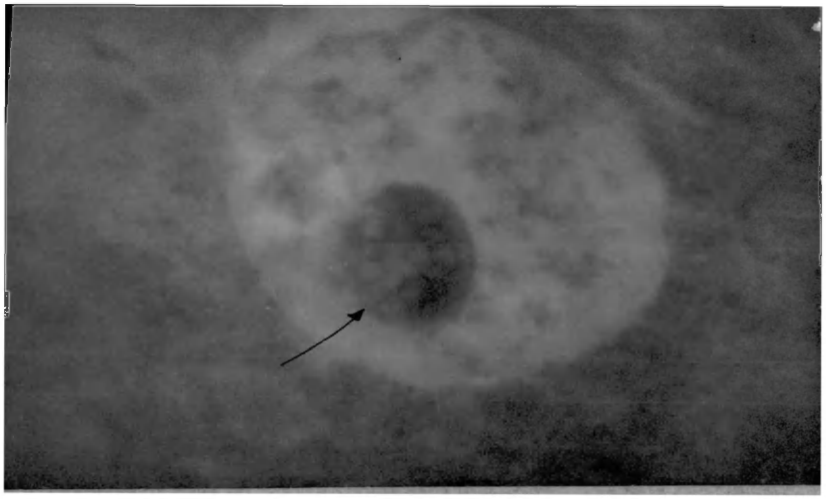


FIG. 18.

Oögonium, 101 mm. larva, showing nuclear structure.

From 1,455X

Text reference: p. 40

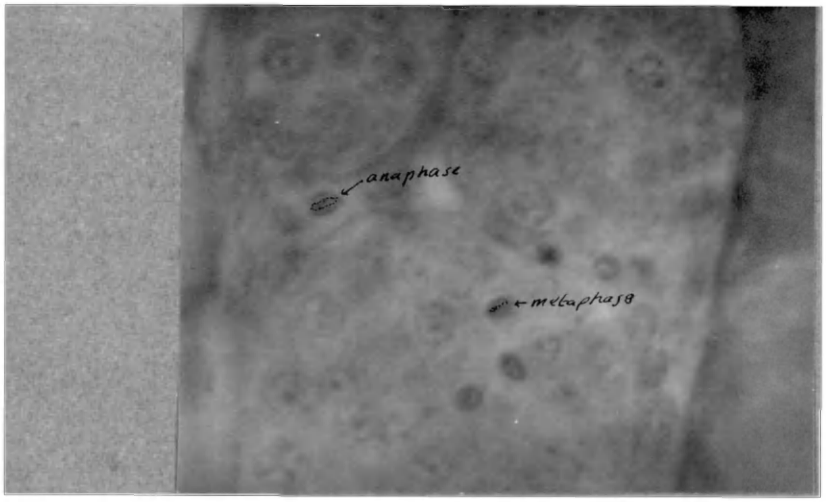


FIG. 19.

Spermatogonia, 53-1/2 mm. larva.

From 970X

Text reference: p. 41

PLATE
X

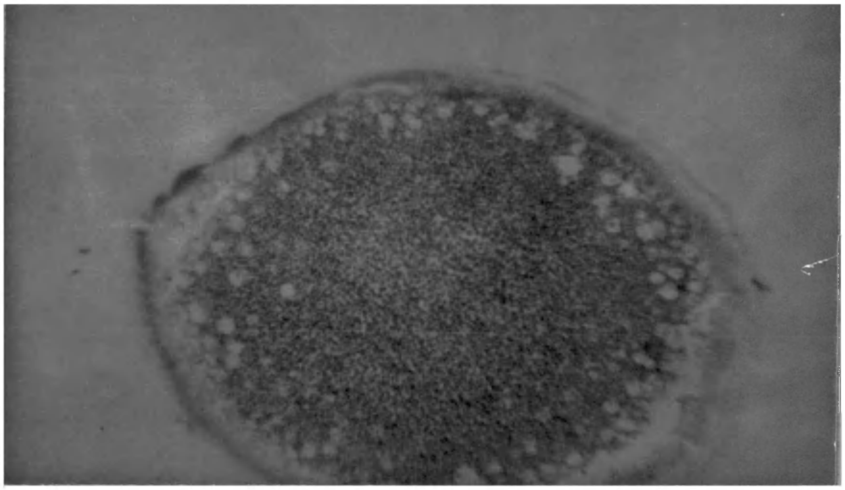


FIG. 20.

Oögonium, 99 mm.
larva.

From 100X

Text reference:
p. 42

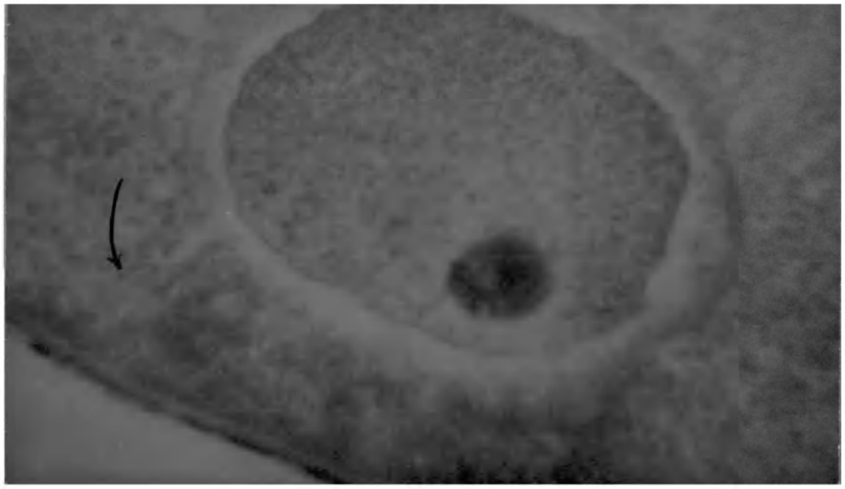


FIG. 21.

Nucleus of oögonium,
99 mm. larva

From 1,455X

Arrow points to polar
cytoplasm

Text reference:
p. 42

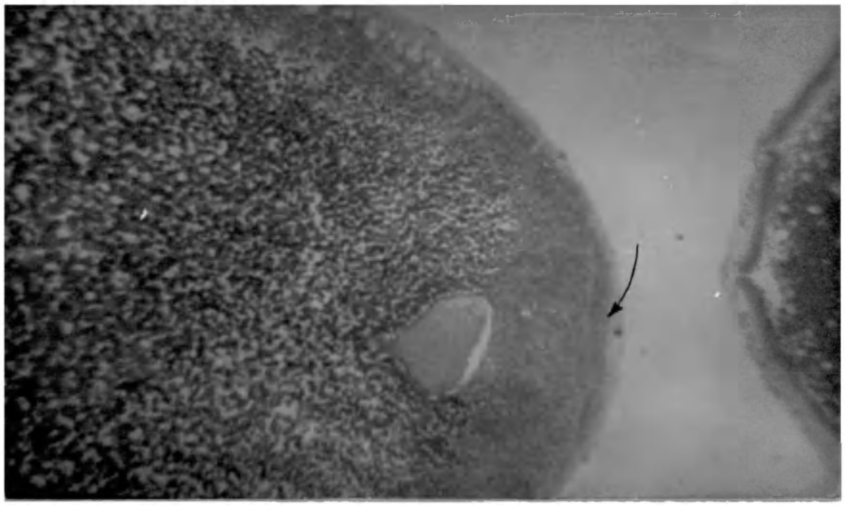


FIG. 22.

Oogonium, about
100 mm. larva

From 100X

Text reference:
p. 44

Arrow points to
polar cap

PLATE
XI

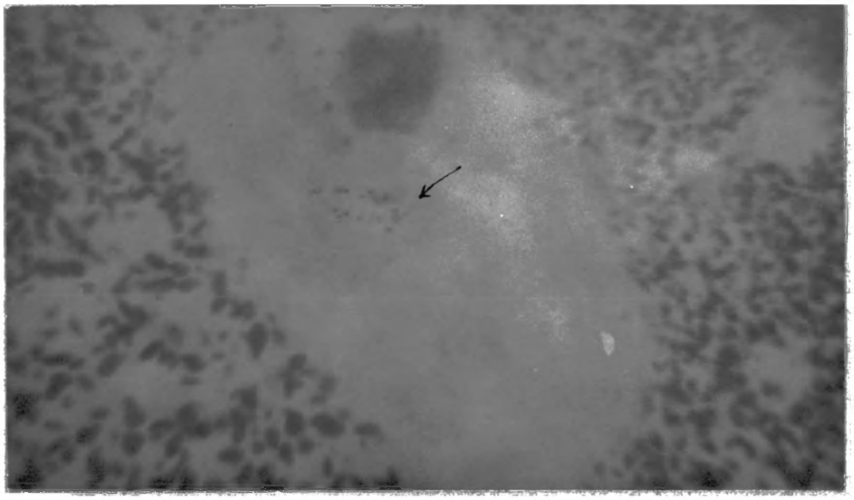


FIG. 23.

Primary oöcyte,
about 100 mm.
larva

From 970X

Arrow points to early
anaphase of
Meiosis I

Textreference:
p. 44

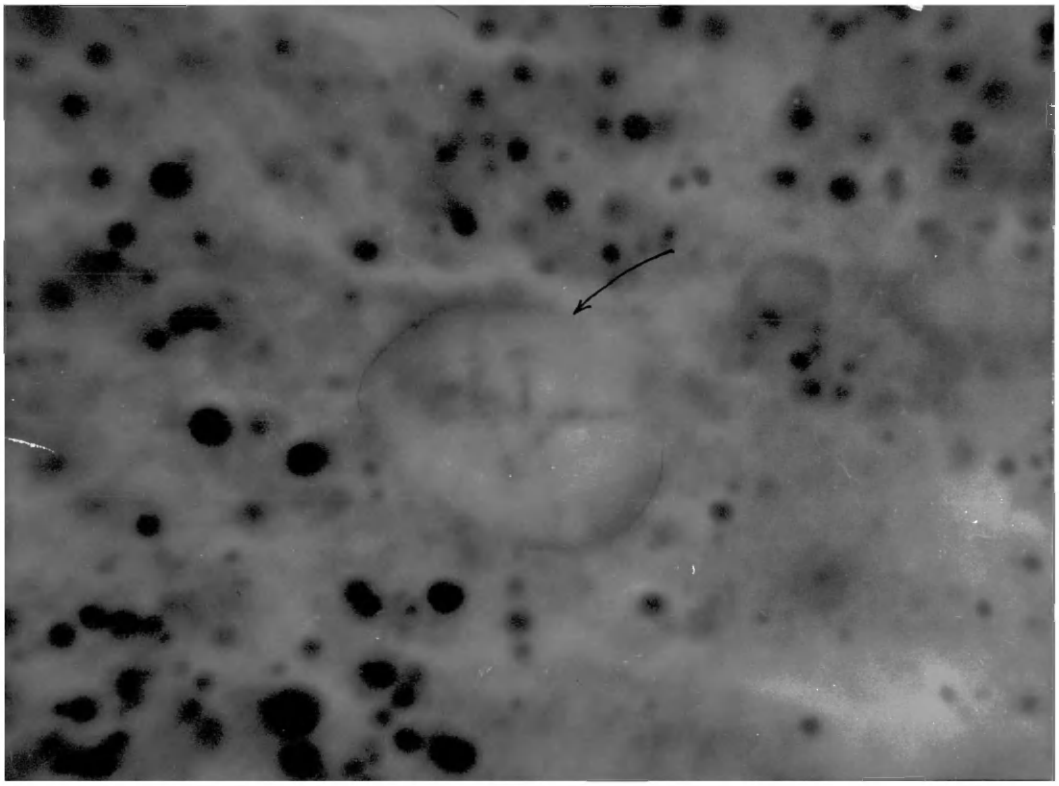


FIG. 24.

Secondary oöcyte in 118 mm. larva. From 1,455X times 4
Arrow poits to nucleus.

Text reference: p. 45

PLATE
XII

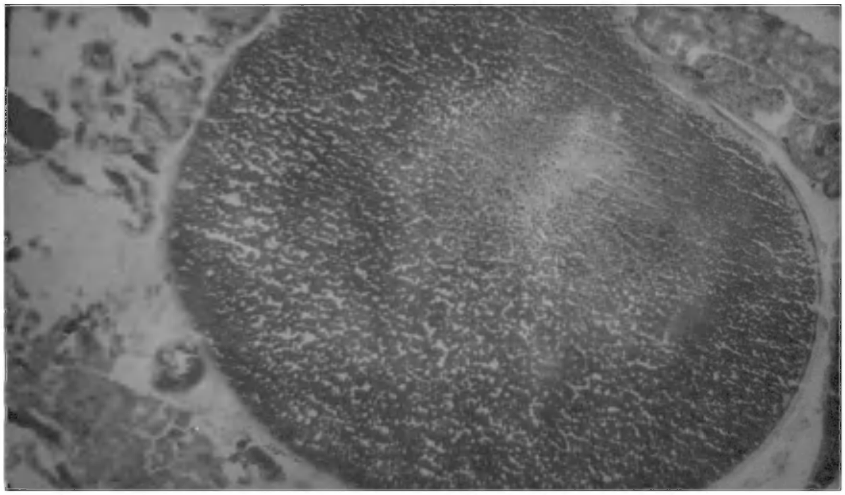


FIG. 25.

Secondary oöcyte (unlayed egg, *in situ*), from gravid adult.

From 100X

Text references: pp. 46 and 47

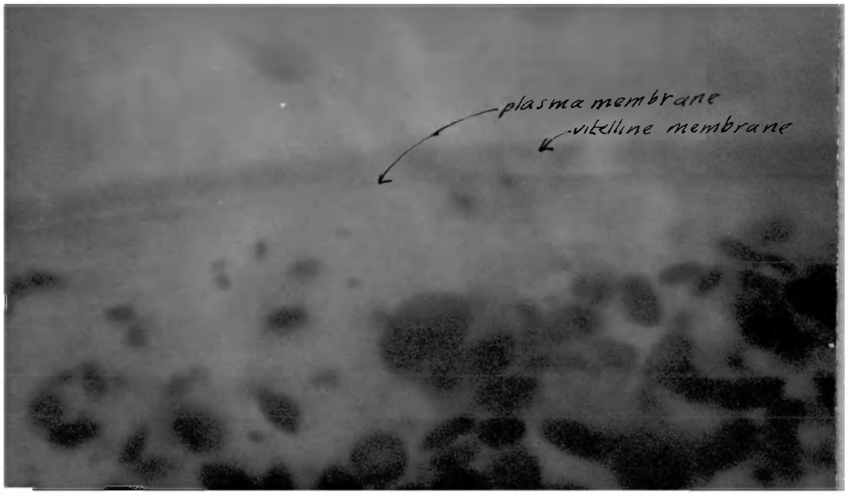


FIG. 25a.

Secondary oöcyte, structure of vitelline membrane.

From 1,455X

Text reference: p. 46

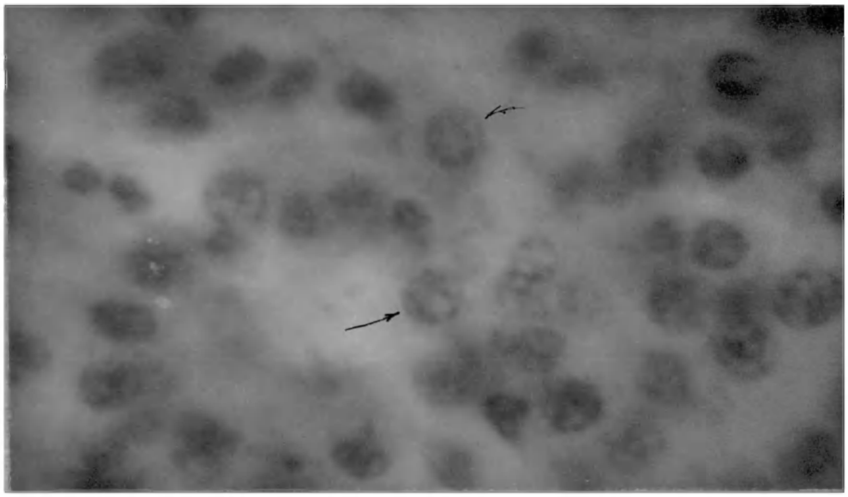


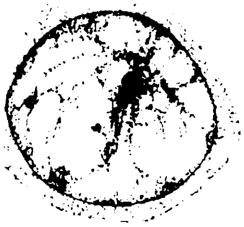
FIG. 26a.

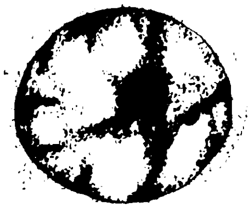
Primary spermatocytes in testis of 116-1/2 mm. metamorphosing animal

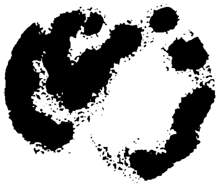
From 1,455X

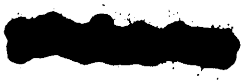
Chiefly prophases

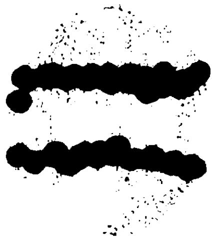
DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.). PLATE XIII









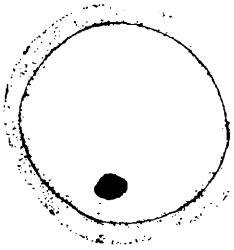


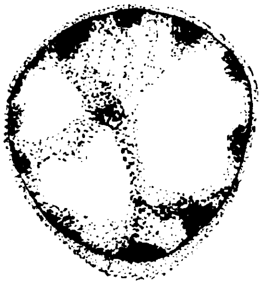
MEIOSIS I in the primary spermatocyte, 116-1/2 mm. metamorphosing animal. Drawn from 1,455X and enlarged to about 4,000X

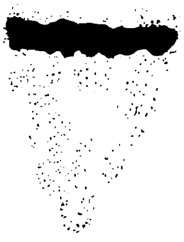
FIG. 26b. Metabolic phase. Text reference: p. 48
FIG. 27. Zygotene? stage. Text reference: p. 49
FIG. 28. Pachytene stage. Text reference: p. 49
FIG. 29. Diakinesis. Text reference: p. 50
FIG. 30. Metaphase. Text reference: p. 50
FIG. 31 Anaphase. Text reference: p. 50

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (*LAMPETRA AEPYPTERA*, sup.).

PLATE
XIV





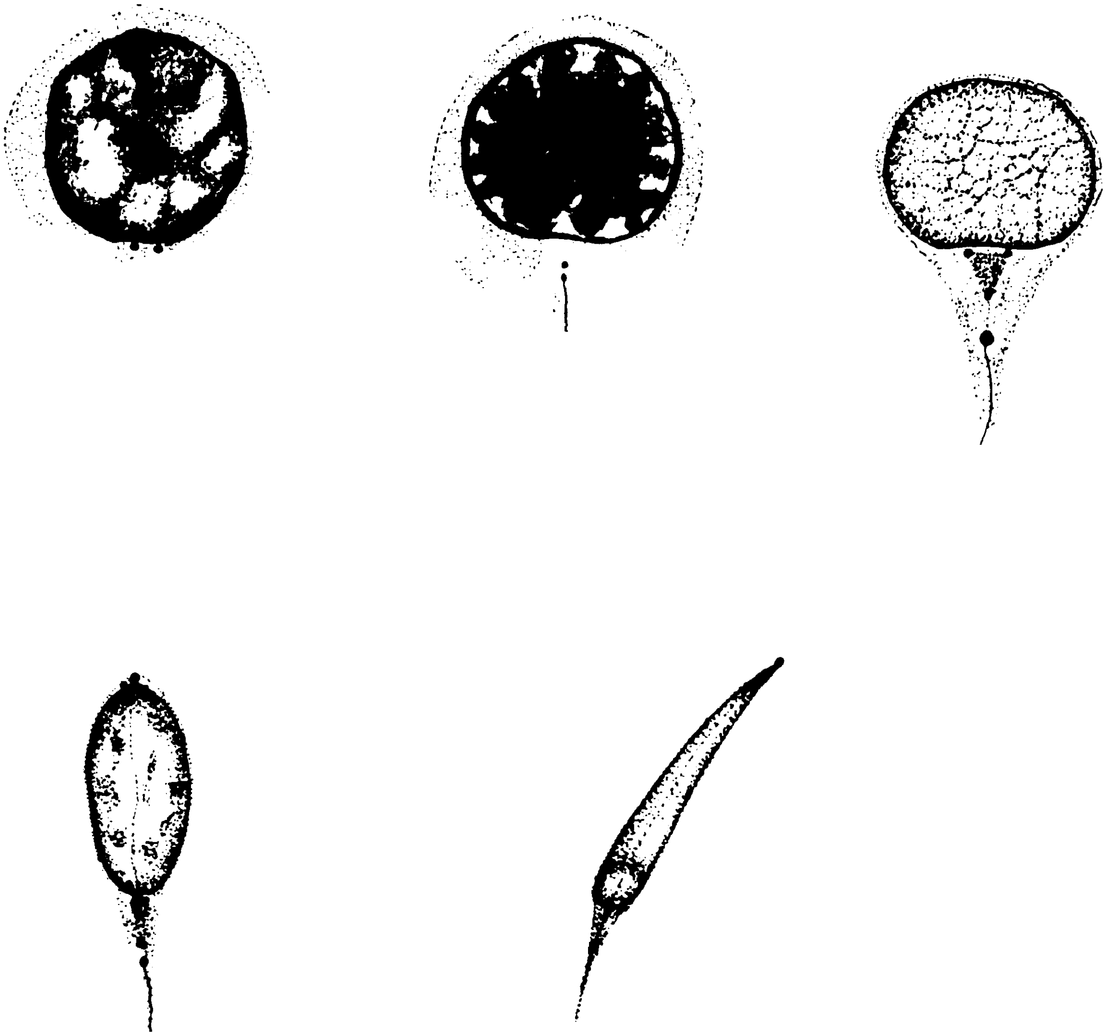


MEIOSIS II, secondary spermatocyte, 101 mm. metamorphosing animal. Drawn from 1,455X and enlarged to about 4,000X

FIG. 32. Metabolic phase. Text reference: p. 50
FIG. 33. Prophase. Text reference: p. 51
FIG. 34. Metaphase. Text reference: p. 52

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE
XV



SPERMIOGENESIS in a 110 mm. metamorphosing animal. From 1,455X

FIG. 35: incipient stage. From 1,455X to about 4,000X. Text references: pp. 54, 55. In FIG. 36 and FIG. 37, development of tail filament (4,000X); in FIG. 38 and FIG. 39, shaping of the sperm head (3,000X).

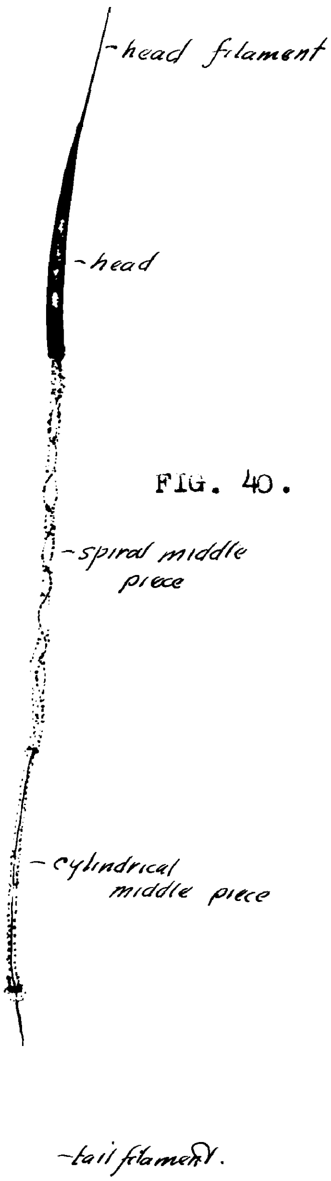

head filament
head
spiral middle piece
cylindrical middle piece
tail filament.

FIG. 40.

FIG. 40. Spermatozoon.

From 1,455X to about 2,000X.

Text reference: p. 57

PLATE XVII

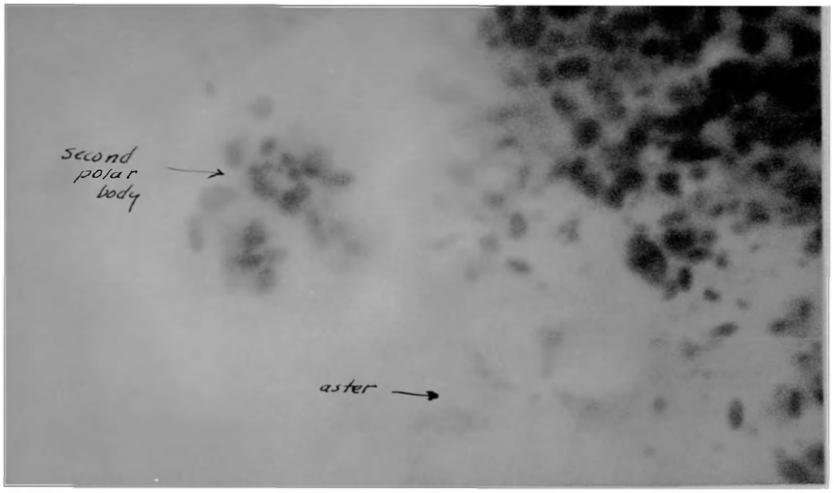

Second polar body
aster

FIG. 41.

Extrusion of second polar body.

From 1,455X times 2

Text reference: p. 61

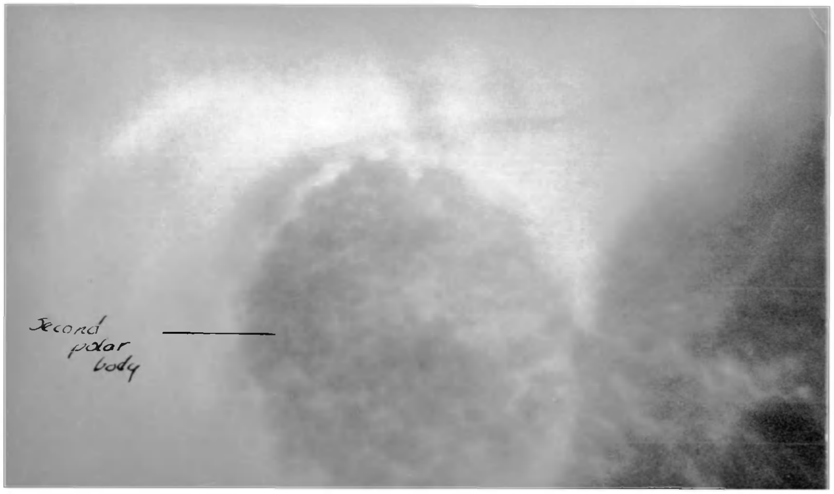

Second polar body

FIG. 42a.

Second polar body completely extruded from fertilized egg.

From 1,455X times 2

Text reference: p. 61.

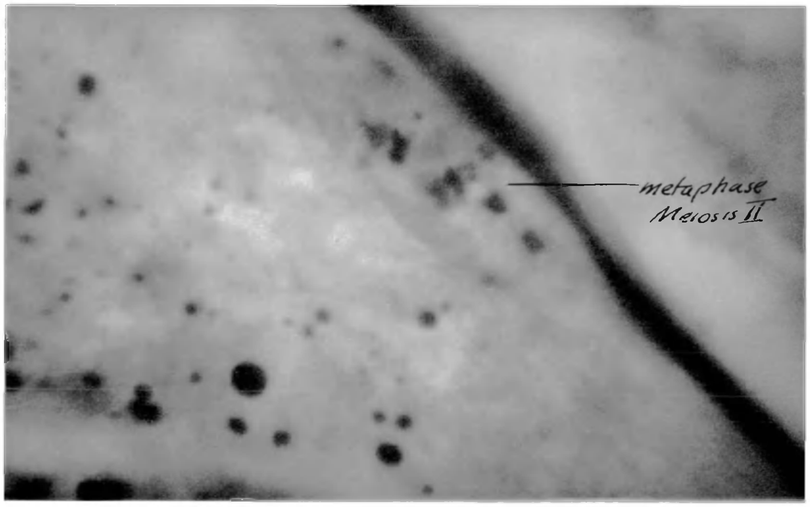

metaphase Meiosis II

FIG. 42b.

Metaphase of Meiosis II

From 1,455X times 2

Text reference: p. 61

PLATE XVIII

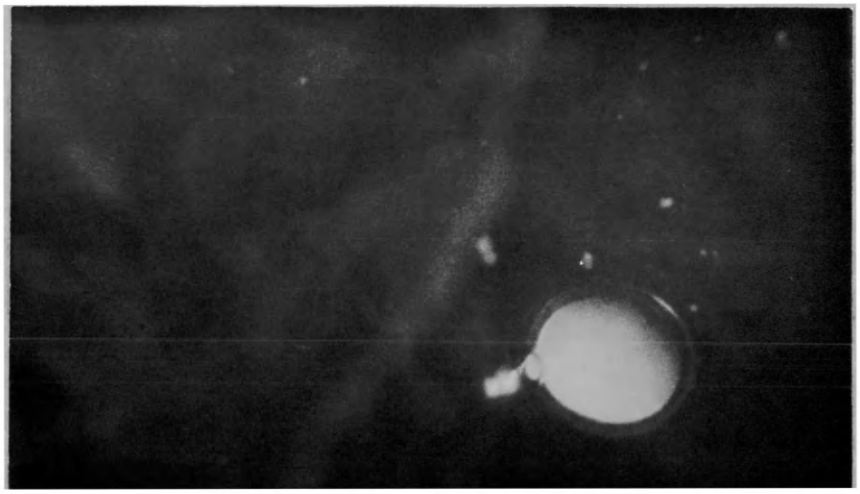


FIG. 42c.

Fertilized egg, external view

About 10X

Text reference: p. 62

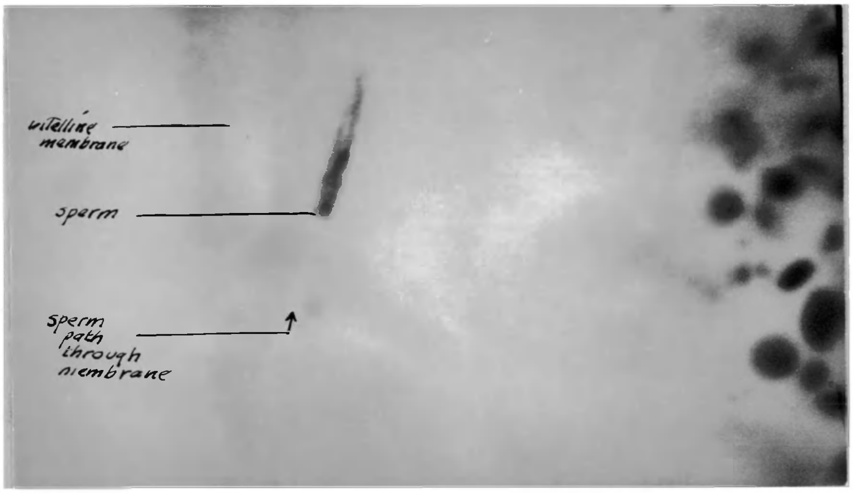

vitelline membrane
sperm
sperm path through membrane

FIG. 43.

Penetration of sperm head into egg

From 1,455X to about 2,500X

Text reference: p. 62

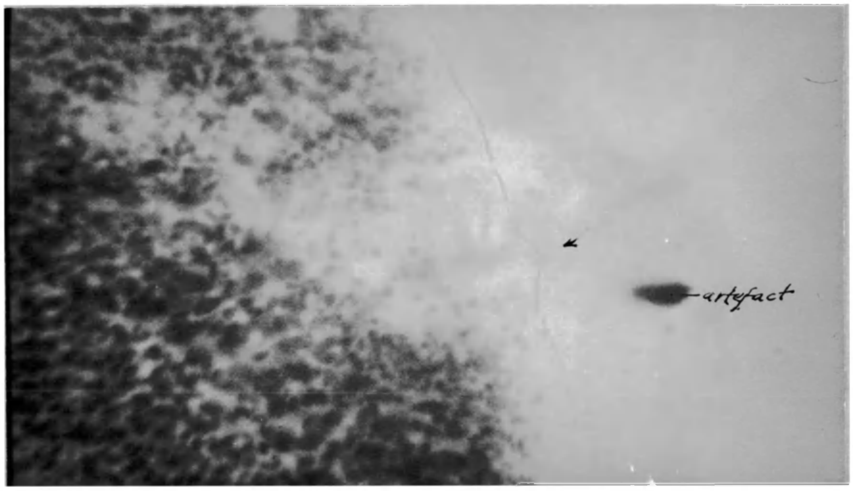


FIG. 44.

Sperm entrance papilla

From 970X times 2

Text reference: p. 62

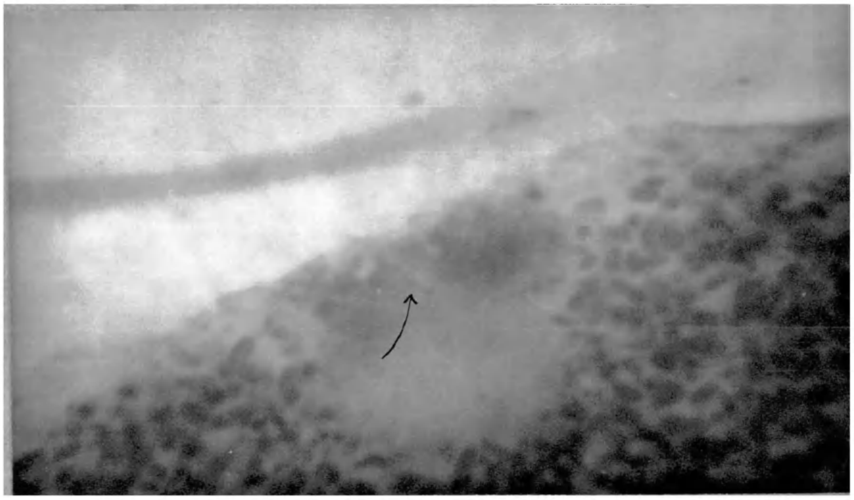


FIG. 45.

Rotation of sperm head. Arrow points to sperm head.

From 1,455X

Text reference: p. 64.

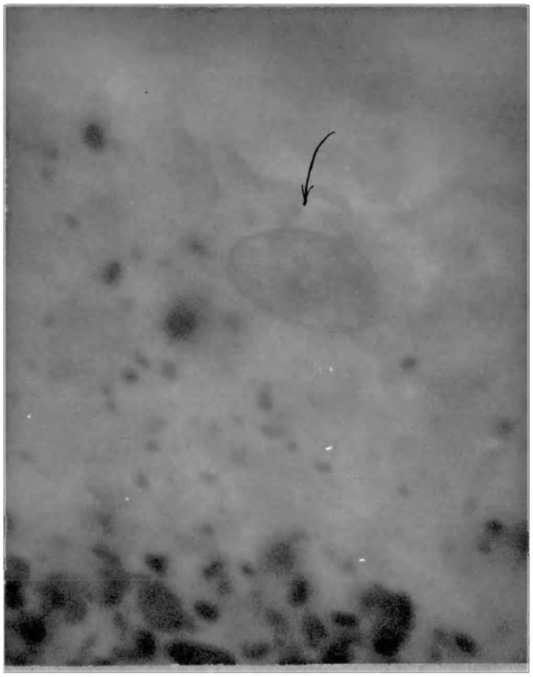


FIG. 46a.

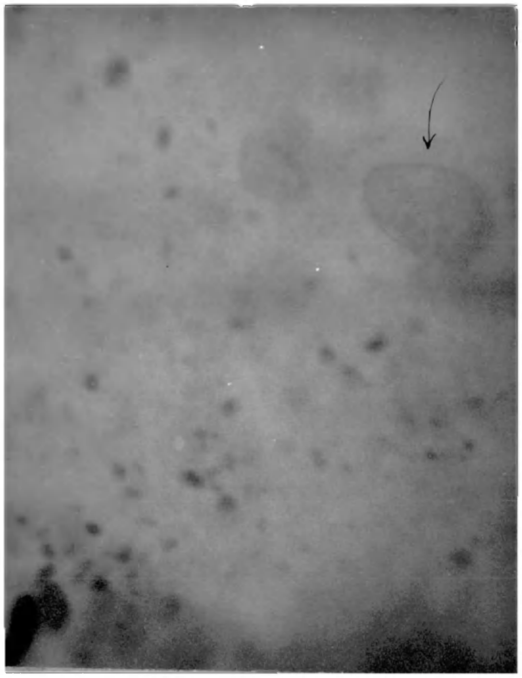


FIG. 46b.

Pronuclei approaching each other for the first cleavage metaphase. These two figures were on succeeding sections, and one belongs to one pronucleus and the second, to the other.

From 1,455X

Text reference: p. 65

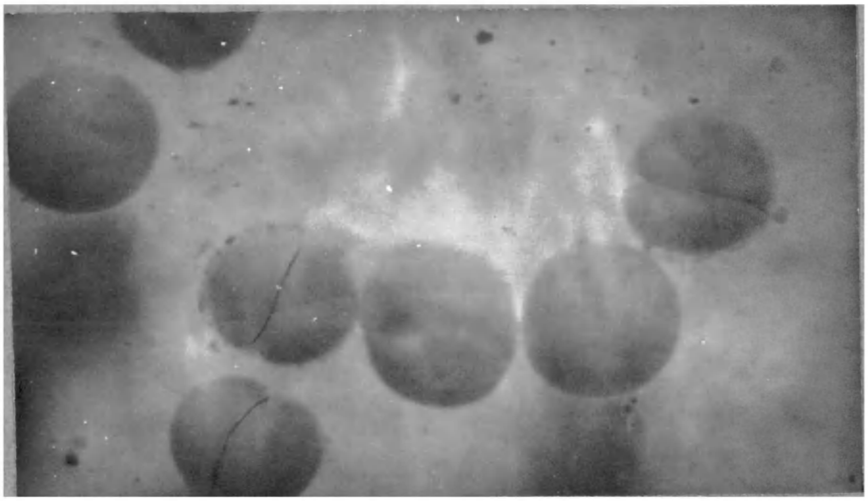


FIG. 47.

First cleavage

About 15X

Text reference: p. 67

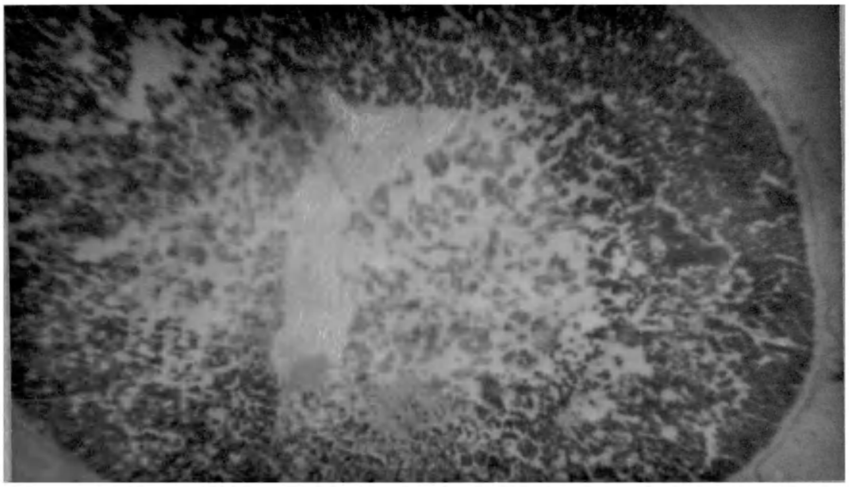


FIG. 48.

Cleft between blastomeres, first to second cleavages

From 100X

Text reference: p. 68

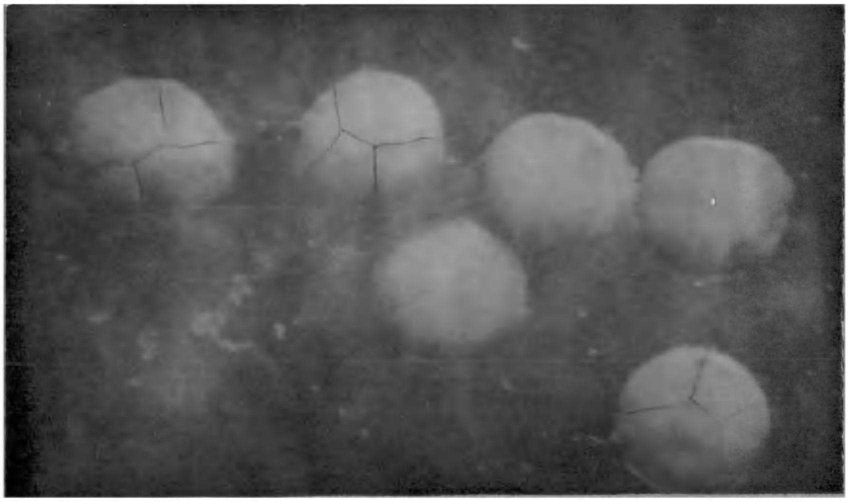


FIG. 49.

Second cleavage

From about 15X

Text reference: p. 68

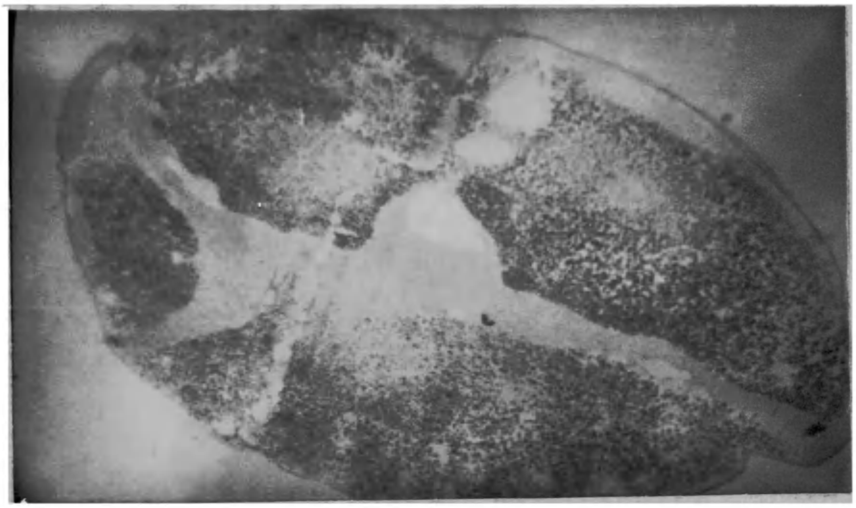


FIG. 50.

Cross-section of second cleavage.

From 100X

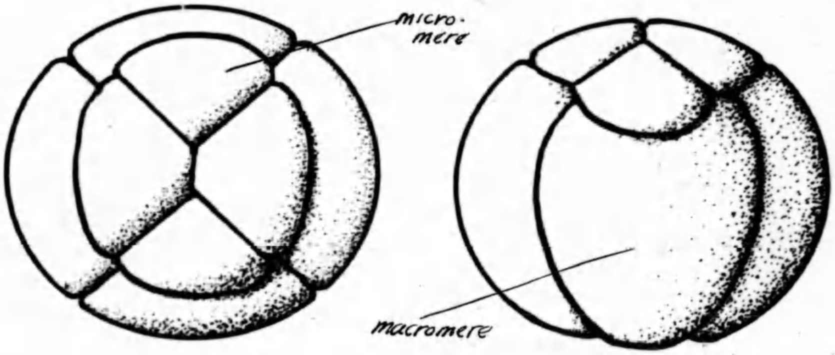
Text reference: p. 70

FIG. 51.

Third cleavage.

From 15X

Text reference: p. 72



FIGS. 52a and 52b.

Third cleavage, from dorsal and lateral aspects.

About 50X

Text reference: p. 72

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE
XXII

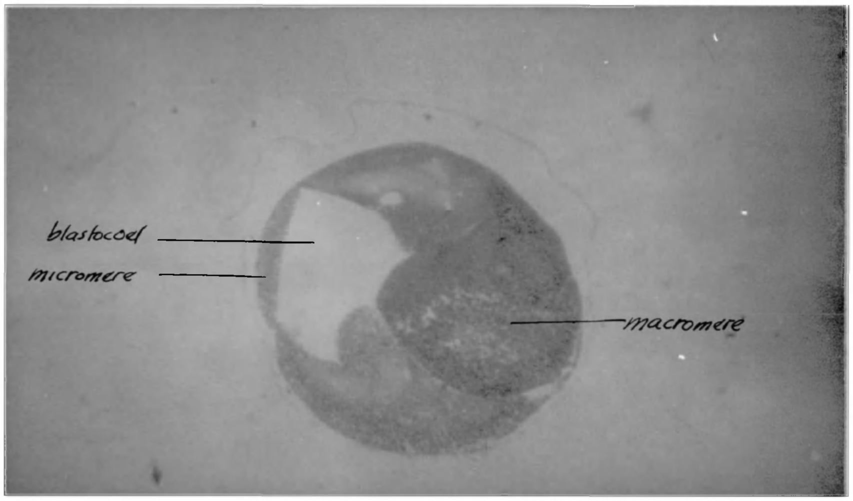

blastocoel
micromere
macromere

FIG. 53.

Third cleavage: blastocoel

From 50X

Text reference: p. 83

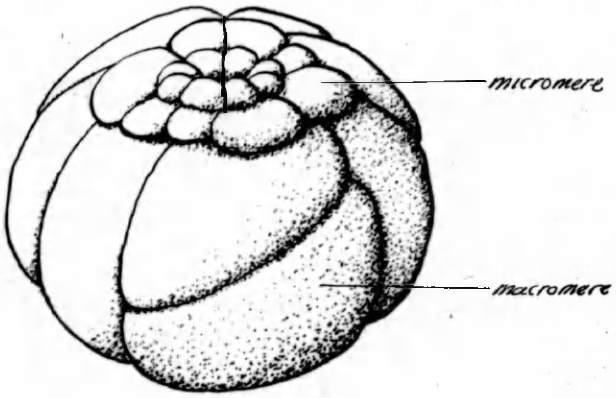

micromere
macromere

FIG. 54.

Very early blastula

About 50X

Text reference: p. 83

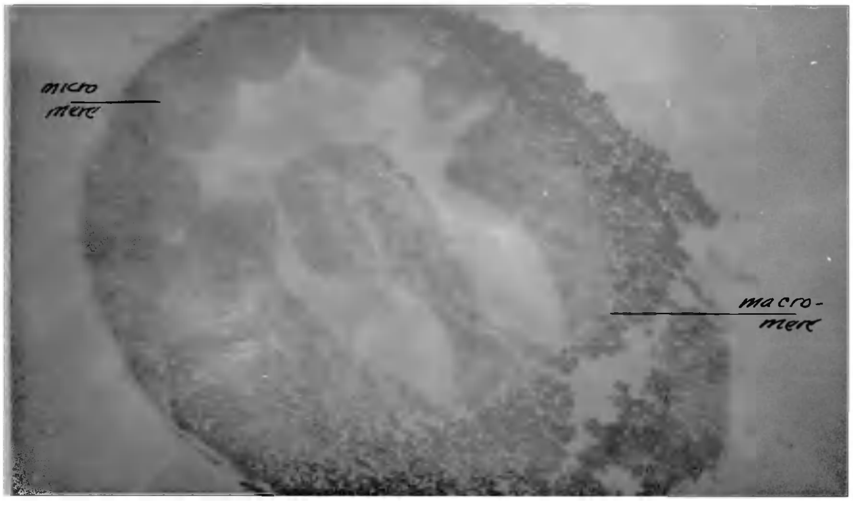

micro mere
macro-mere

FIG. 55.

Blastula: micromeres in one row

From about 100X

Text reference: p. 83

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE
MARYLAND BROOK LAMPREY (*LAMPETRA AEPYPTERA*, sup.).

PLATE
XXIII

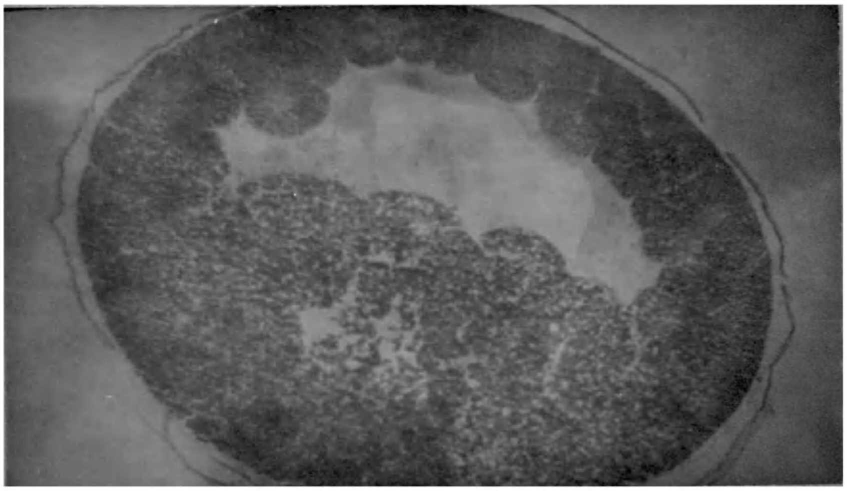


FIG. 56.

Blastula, micromeres in two rows

From 100X

Text reference,
p. 84

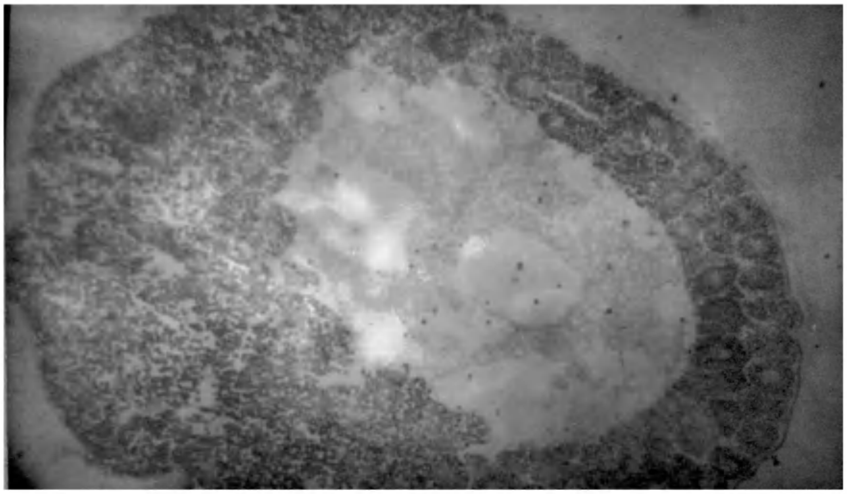


FIG. 57.

Blastula, micromeres in three rows

From 100X

Text reference:
p. 84

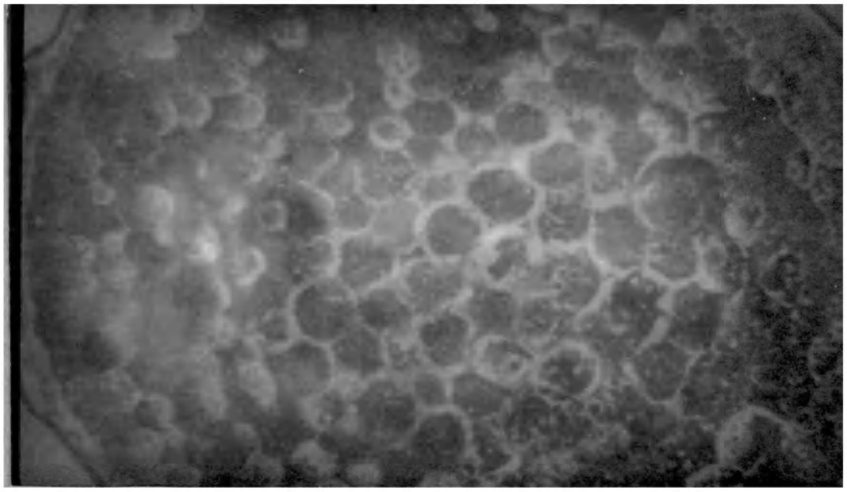


FIG. 58.

Lateral section through animal pole, blastula having four layers of micromeres

From 100X times 2

Text reference:
p. 84

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (*LAMPETRA AEPYPTERA*, sup.).

PLATE XXIV

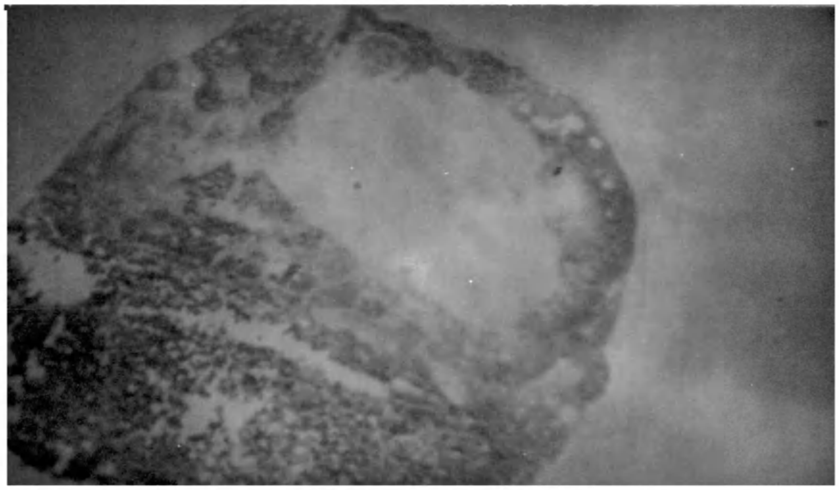


FIG. 59.

Post-blastula, beginning of micromeric migration
From 100X

Text reference:
p. 87

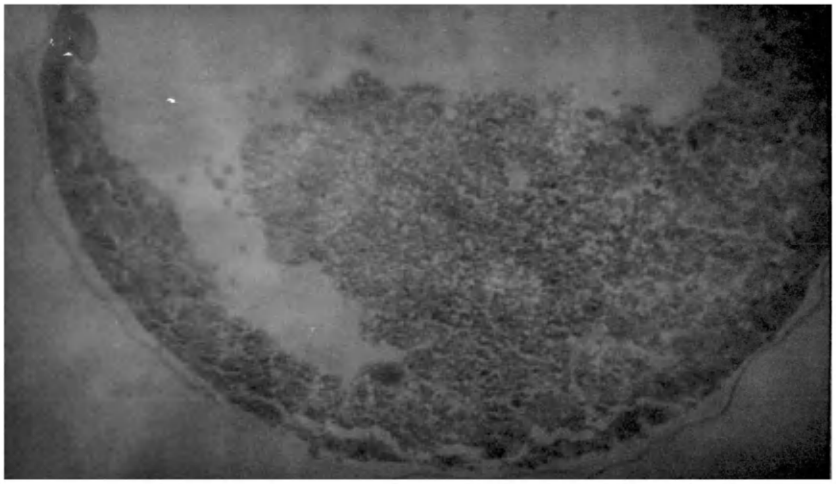


FIG. 60

Late post-blastula

From 100X

Text reference:
p. 87

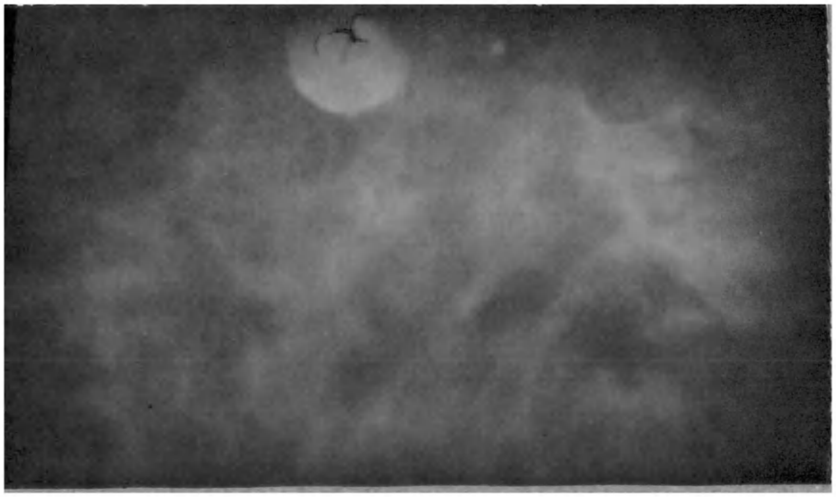


FIG. 61.

Torula

About 15X

Text reference:
p. 88

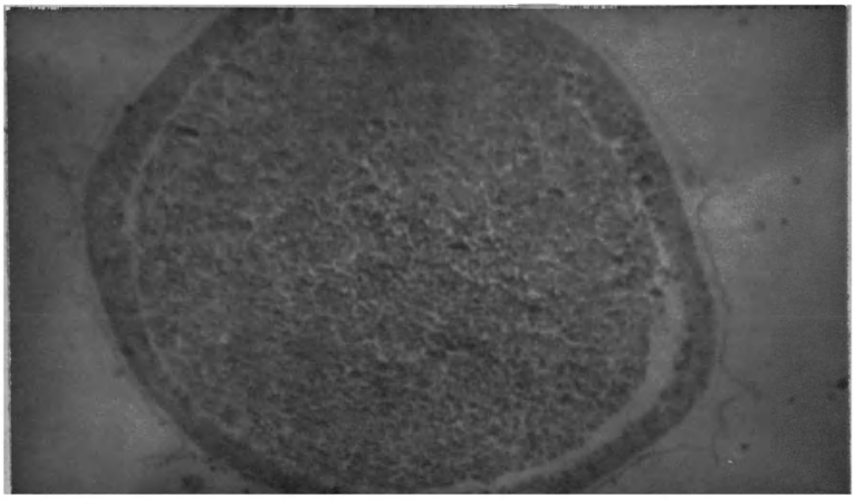


FIG. 62.

Early gastrula, section

From 50X times 2

Text reference: p. 90

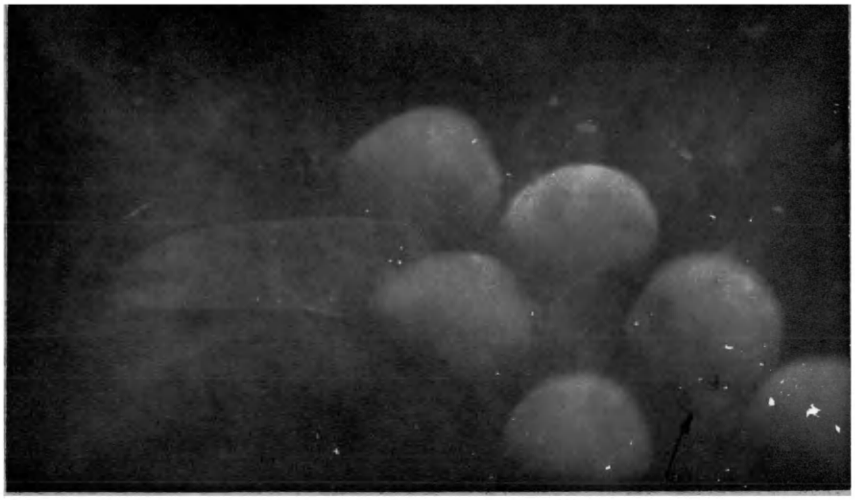


FIG. 63.

Early gastrula, external view

About 15X

Text reference: p. 90

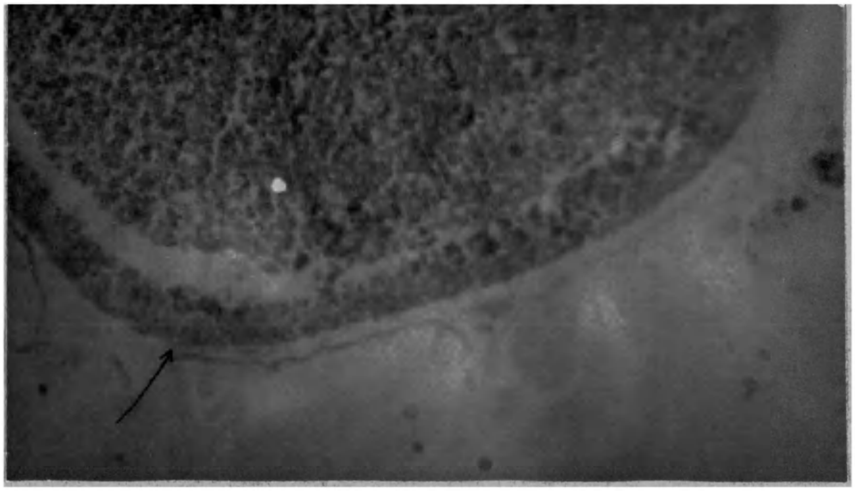


FIG. 64.

Early gastrula, ectodermal epithelium

From 100X times 1.5

Text reference: p. 91

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE XXVI

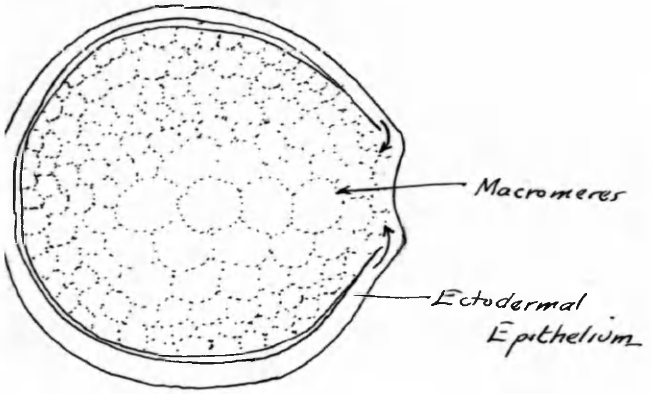


FIG. 65.

Gastrula: disposition of macromeres

About 50X

Text reference: p. 92

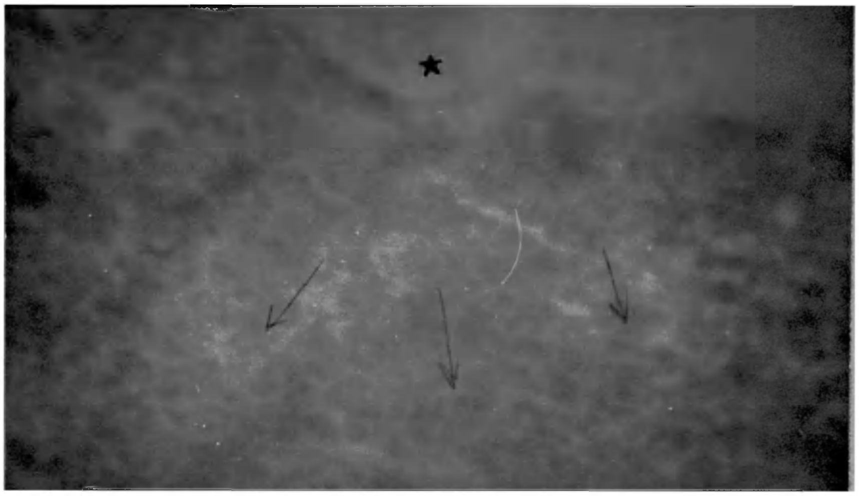


FIG. 66.

Involution at the blastopore. * indicates lip and arrows, lines of flow

From 970X

Text reference: p. 93

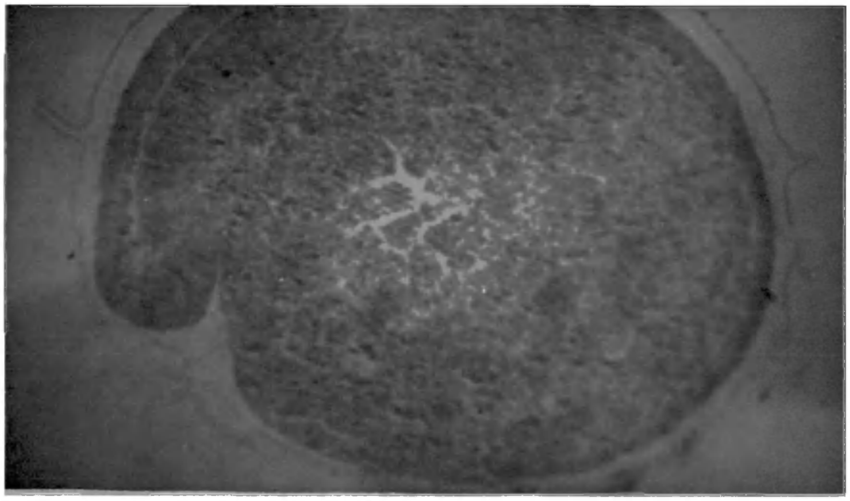


FIG. 67.

Gastrula

From about 100X

Text reference: p. 93

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE XXVII

FIG. 68.

Transitional area in germ layers, dorsal lip

From 970X

Text reference: p. 94

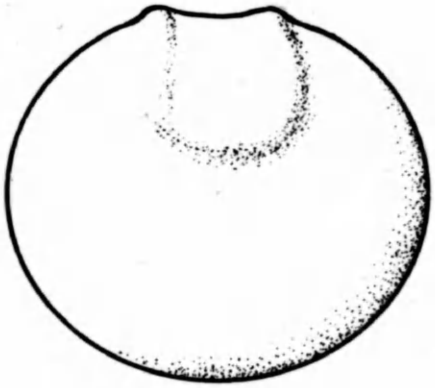


FIG. 69.

Early neurula

About 50X

Text reference: p. 99

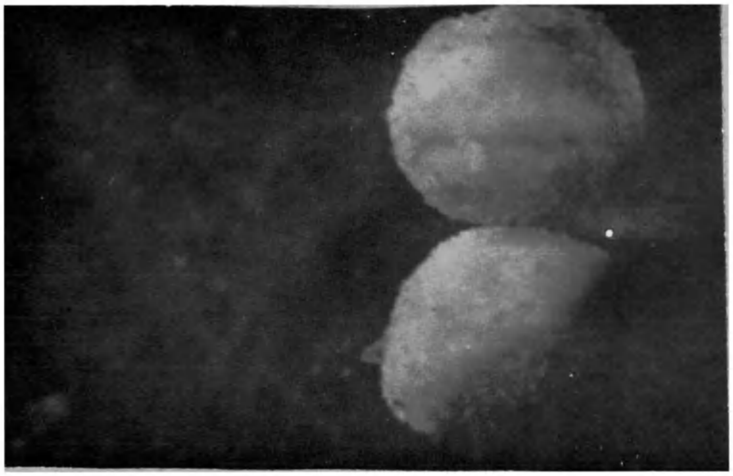


FIG. 70.

Neurulae

About 15X

Text reference: p. 99

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.).

PLATE XXVIII

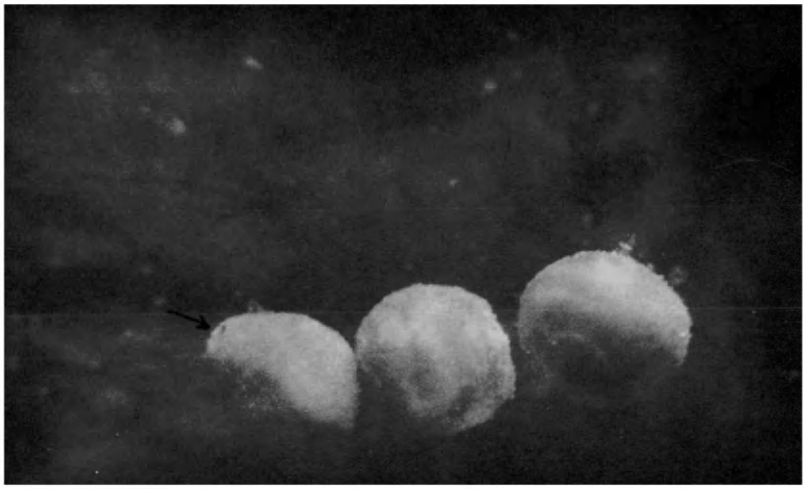


FIG. 71.

Neurulae. Arrow points to blastopore.

About 15X

Text reference: p. 100

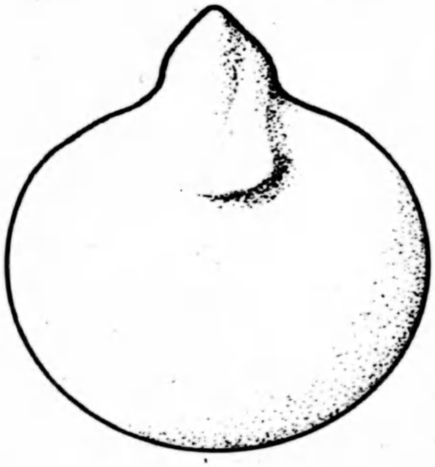


FIG. 72.

Late neurula, with pronounced reural ridge. Head end.

About 50X

Text reference: p. 100

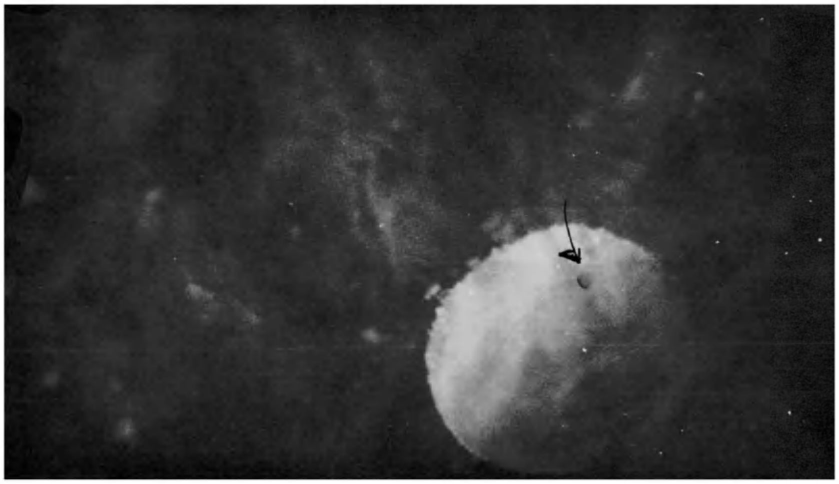


FIG. 73.

Neurula: blastoporal slit

About 30X

Text reference: p. 100

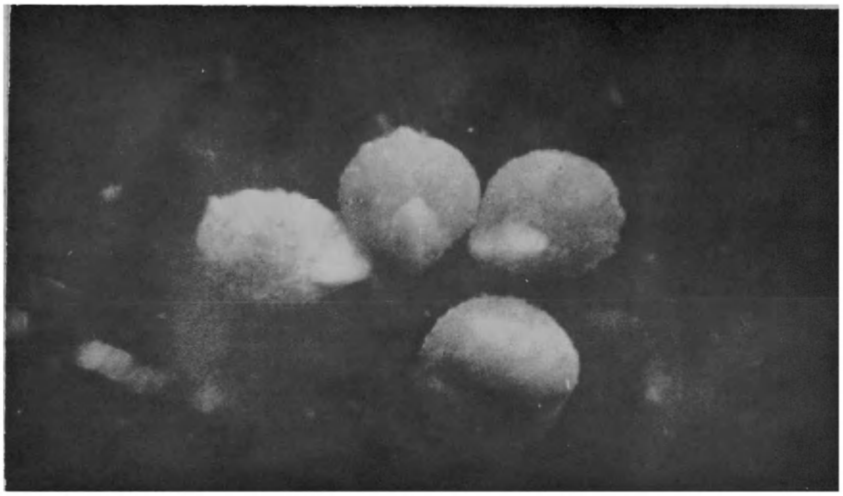


FIG. 74.

Embryo, early head extension

About 15X

Text reference: p. 101

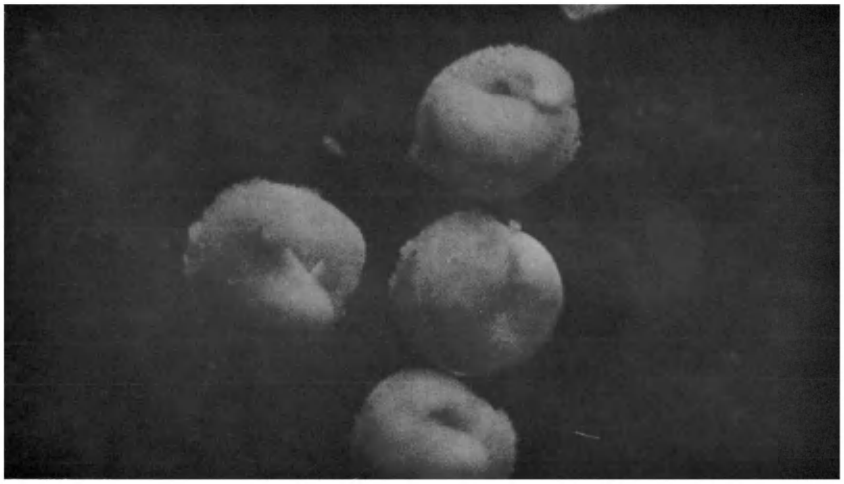


FIG. 75.

Embryo, middle head extension

About 15X

Text reference: p. 102

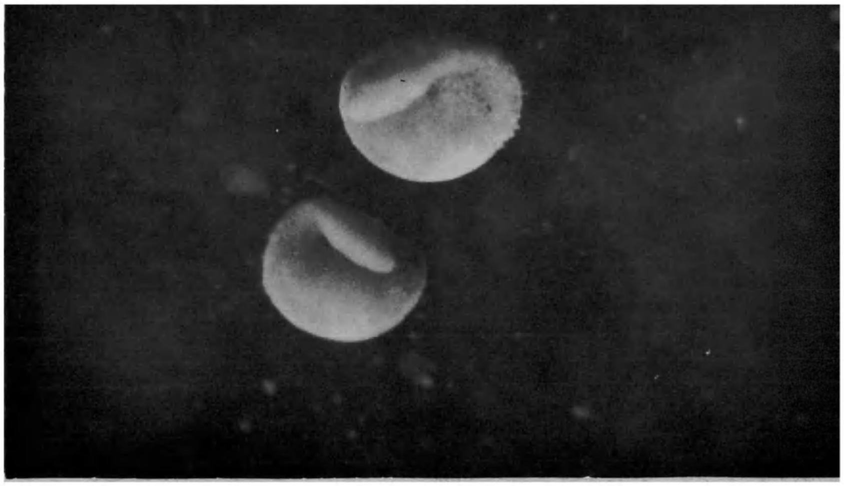


FIG. 76.

Late head extension

About 15X

Text reference: p. 102

PLATE
XXX

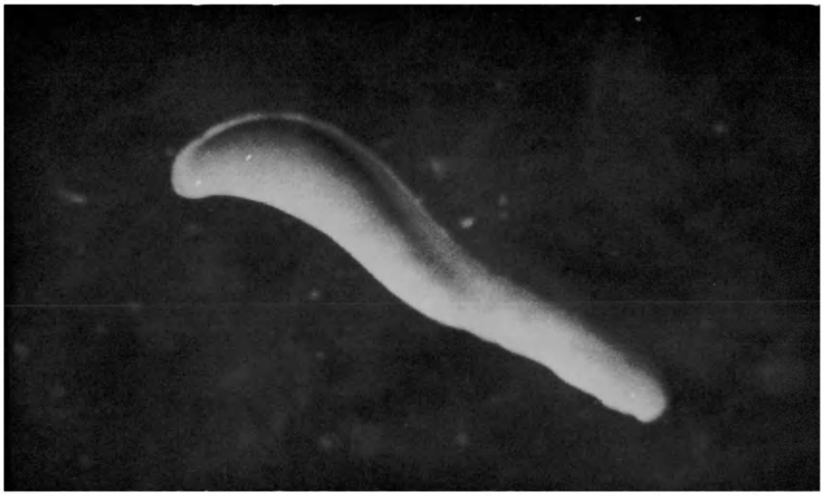


FIG. 77.

Larva, at hatching

About 15X

Text reference:
p. 103

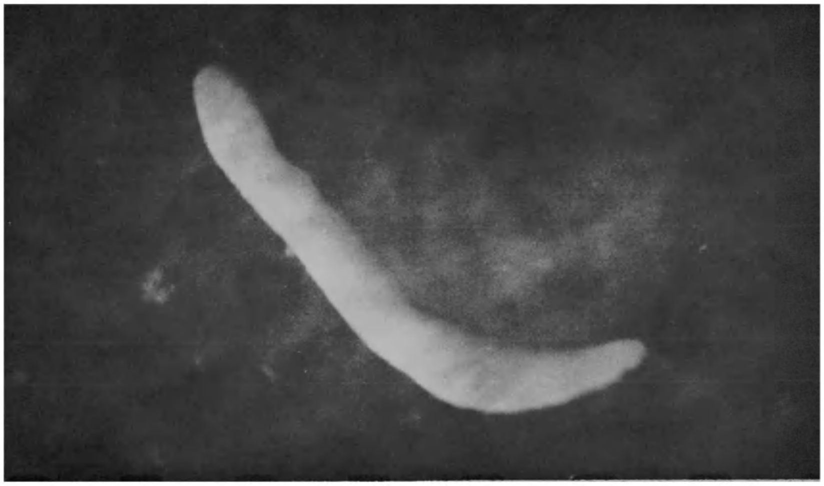


FIG. 78.

Larva, first week

About 15X

Text reference:
p. 103

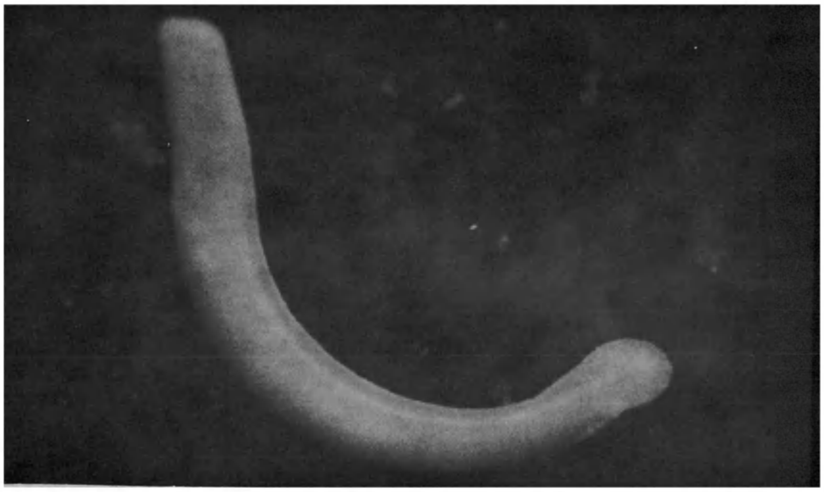


FIG. 79.

Larva, first week

About 15X

Text reference:
p. 103

SUPPLEMENT

DEVELOPMENT OF THE GAMETES AND EARLY EMBRYO IN THE MARYLAND BROOK LAMPREY (LAMPETRA AEPYPTERA, sup.)

by

Herbert F. Seversmith

LIBRARY
UNIVERSITY OF MARYLAND
COLLEGE PARK, MD.

1953

(Supplement to original thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1952)

ACKNOWLEDGEMENTS

The writer has been greatly aided by the advice given by Dr. William R. Duryee of the National Cancer Institute, Bethesda, Maryland, and by his expert micro-dissection of the germinal vesicle of *Lampetra aepyptera*. Thanks are also given to Messers. William E. Maloney of Washington, D. C., and Harold Muma of College Park, Maryland, for help in collection of material.

211991

INTRODUCTION

On page 37 of the thesis, under Development of the Oögonium, it is stated that the cytoplasm of the first year oögonium is divided into two well-defined areas: a hyaline, ovaloid central area about 25.8 u in diameter in which a darkly staining round body of 8.6 u in size is eccentrically located, and a very finely granular field which is identified as the yolk area. It is stated further that the circumscribed hyaline area had been identified in other brook lampreys as the nucleus, but in the present case it was considered to be cytoplasm, and then it would follow that the smaller, darkly stained round body would be the nucleus and not a nucleolus.

Two considerations influenced the writer to this conclusion: (1) the observation of Lubosch ('04) that the chromosomes issued from the "nucleolus" and (2) the extraordinary size that the so-called nucleolus would attain; and to this might be added that the metaphase chromosomes of Meiosis I formed a plate which could be placed almost entirely within the area occupied by the "nucleolus".

Nevertheless, the writer has not been happy about this situation, and recently an opportunity was presented to dissect the germinal vesicle in vivo by the micro-dissection techniques of Duryee ('37, '41), in order to determine the actual size and relationships of the germinal vesicle. This has been done, and it is found that in this respect the writer's work needs correction.

The observations which follow are based upon these recent experiments, and the discussion which follows is a revision and extension of that hitherto presented.

On page 81 there were three phases listed under discussion of the blastula: the blastula, the post-blastula and the torula. While the writer believes that there is nothing intrinsically wrong with these concepts, most writers consider that the stage described as the post-blastula actually initiates gastrulation, Accordingly revisions have been made.

METHODS

In vivo microdissection of the developing egg was performed according to the methods of Duryee ('37, '41) to isolate and characterize the germinal vesicle.

OBSERVATIONS

The Oögonium in First Year Larvae

(revision of original thesis, pages 37-38)

The field of the germ cell is divided into three well-defined areas: a more or less ovaloid central area about 25.7 x 33.2 u, in which a darkly staining, spherical body having a diameter of 8.6 u is eccentrically located; and a very finely granular field which is destined to be the deutoplasm. The granules of the last stain deeply. Microdissection in vivo of second-year larvae shows that a membrane separates the deutoplasm from the lighter area; the latter is therefore considered to be the nucleus or germinal vesicle. The smaller, more darkly-staining body within the nucleus has been termed a nucleolus in the literature, but its true character remains to be determined.

As has been stated, there appears to be a great and somewhat sudden increase in the size of the oögonium about this time. From the appearance of the deutoplasm, the writer is of the opinion that the apparently very rapid augmentation in volume of the deutoplasm is not attained by intrinsic growth alone. The ovum is regarded as the "successful" cell in a cluster of cells (those of the cyst or follicle of other writers), and its deutoplasm appears to be contributed, at least in part, by such accessory cells. The progression of these stages has not been seen in vivo; the sequence of stages available at this time can only suggest but not conclusively prove that such is the case. The cytoplasm of these accessory cells becomes very granular, and it has practically the same appearance which is characteristic of the developing deutoplasm of this stage.

The Oögonium in Second-Year Larvae

(revision of original thesis, pages 40-41)

On March 29 the ova of a second-year larva were examined in the living state. These eggs had attained cross-axes of 190 x 150 u; the germinal vesicle had an average diameter of 75 u, and the so-called "nucleolus" was from 25 to 33 u in diameter. The deutoplasm was quite translucent, and the gross structure of adjacent eggs could be seen without much difficulty through it. The germinal vesicle was more refractile and except as will be described, was quite uniformly hyaline. The "nucleolus" was likewise fairly transparent, and it had the highest index of refraction; under dark-field illumination it shone brilliantly.

A number of the nuclei of these ova were dissected from these ova and studied. Whereas in the intact egg the form of the germinal vesicle was frequently ovoid, and its borders apparently somewhat difficult to establish in fixed material, upon being freed from the deutoplasm the nucleus became a sphere, or nearly so. Its membrane was fully apparent as an extremely thin structure whose thickness was less than 0.5 u. The so-called "nucleolus" maintained its shape and appearance.

In such nuclei, stabilized and stained with crystal violet, very thin, attenuated structures of varying lengths became apparent; it is believed that these are the chromonemata. At the same time the "nucleolus" absorbs the stain quite markedly, and in one such preparation wherein the nucleus was ruptured, the escaping "nucleolus" maintained its form during a short period of observation.

The Oogonium in Third-Year Larvae and the Oocytes

(revision of original thesis, pages 41 ff.)

Except for growth, the oögonium does not change materially in appearance until the fall. In October of the third year the ovum of the larva of 99 mm. length has a variable but somewhat ellipsoidal form; the average diameter of a circle of equivalent area in midsection would be 385 u. On October 13 the future egg exhibits a deutoplasm in which the larger yolk granules have attained dimensions of about 4.3 x 5.3 u; and these begin to resemble the yolk granules of the mature egg. The granules are distributed evenly throughout the egg except at the periphery, where a circumferential series of frothy vesicles begins to appear. These vacuoles range up to about 8 u in diameter and foreshadow the advent of maturation.

The background of the germinal vesicle has now become more apparently granular and stains more deeply. The cross axes are about 65 x 104 u. The nucleus is oriented usually to one side of the cell, which may be properly termed a pole of the cell. There is thus created a narrow layer of deutoplasm between the vitelline membrane and the nucleus. This deutoplasm changes character; it becomes denser and contains more finely granulated material which is shaped like a curved band in cross-section.

Concomitant with this change the vacuoles about the periphery of the ovum become much more numerous, and there is at the same time a redistribution of the coarser yolk peripherad, leaving only the finest particles centrally. As the primary oöcyte approaches the time of the first maturation division, the vesicles around the periphery are augmented by new ones appearing throughout the yolk. The nuclear outline is compact and slightly lens-shaped; it has now arrived to within 60 u of the egg membrane. Next to the nucleus there appears the dense cytoplasmic field just referred to, and between the last and the vitelline membrane another structure appears. This is a hyaline, cap-like area, shaped like a watch-glass. It is probably the Deckel of German writers.

The foregoing observations were made in several larvae, but particularly in the oöcytes of a larva of about 100 mm. length fixed on October 8. Five days later another female animal was killed and fixed. In the latter the oöcyte was found to have the characteristically compressed, somewhat polygonal outline of the immature egg; and if converted to circular dimensions, a midsectional plane would have a diameter of about 600 u. The nucleus is now crescentic to lenticular in outline in fixed material, with the nearer side within 60 u of the vitelline membrane, and the long axis is more or less parallel to the latter.

During the steady increment in size of the ovum from the fall of the first year, no division figures have been observed in any oögonium of this species until the first maturation division is initiated in the fall of the third year. That is to say, all mitotic divisions appear to have been accomplished in the first year. This observation is based upon an examination of more than forty series of sections.

The sequence of the several kinetic stages considered to belong to Meiosis I have not been observed in entirety. On October 23 of the third year dividing nuclei are found; the beginning of anaphase is portrayed in Fig. 23. Unexplained is the fact that even at the beginning of the metaphase, a structure appears in the more hyaline field of the nucleus which is of the same size, roughly, as the former supposed "nucleolus", and which stains well. The chromosomes are stocky and short, and are distributed over an equatorial plate which is about 16 u across. The largest chromosomes do not appear to exceed 3.5 u in length and some of them approach 1 u. The cytology of this process in this lamprey is still under investigation, and the PNA and DNA reactions will be checked.

On February 2 a metamorphosing 118 mm. long female was obtained and the ovary dissected. The secondary oöcytes had now achieved dimensions of about 800 x 1,050 u. The somewhat swollen individual chromosomes have been observed near the plasma membrane in a somewhat clear field, which seems to have a well-defined limit, although no membrane can be made out in fixed material. The yolk granules are now coarsest in the center of the egg, and here they attain a size of 8 x 4 u; they are more or less discoidal. Towards the presumptive future animal pole they become somewhat smaller. Near the vitelline membrane the deutoplasmic field is frothy. The nuclear field lies near the presumptive animal pole.

The Blastula

(revision of original thesis, beginning p. 81)

The post-blastula and torula stages are considered to be referable to gastrulation, although some of the development of the post-blastula is clearly intermediate.

DISCUSSION

(augmentation of original thesis, pp. 104 and those following)

Early published work on the development of the germ cells in the brook lamprey is listed in considerable detail by Okkelberg ('21), Lönnberg et al. ('24) and Pietschmann ('33). From numerous accounts there seems to be no doubt that the primordial germ cells are segregated very early in the development of the embryo; but whether or not all, some or any of the definitive, finally developed germ cells are derived from these has been much disputed. The difficulty is, of course, that it is hard to demonstrate this continuity otherwise than *in vivo*, and the present limitations imposed on such observation are obvious. Okkelberg thought that there is a definite continuity of the germ plasm after primary segregation of the cells concerned, and that peritoneal epithelium or other cells do not change into germ cells. With this opinion and from the evidence available in the present case, the writer is in accord. Generally, the primordial germ cells in brook lampreys have not been traced beyond a stage wherein they are located in the posterior part of the body, "probably in a small area around the blastopore" (Okkelberg). Difficulties in determination are augmented by those of establishing the internal migration routes of such cells and also by the lack of criteria which would distinguish the early sex cells from those having other potentialities. In this Maryland brook lamprey, as in *E. lamottenii* (*wilderi*), the peristomal and paraxial regions of the mesoderm are contiguous and do not appear to differ appreciably in structure or in origin. Okkelberg was of the opinion that only the peristomal mesoderm carries the germ cells in early stages; it may well be.

Butcher ('29) found large cells with round, vesicular nuclei in the caudal end of the embryo of *P. marinus unicolor*, and which he considered to be primordial sex cells. He reported likewise that the greater number of these cells lie immediately below the ectoderm ventrolateral to the position of the future pronephric duct. With further differentiation and at the time of hatching, these cells are found in the mesoderm increased in number. They are then situated, for the most part, ventral to the pronephric ducts; this agrees well with the situation as found in the Maryland lamprey. In the latter, however, the germ cells become more clear of yolk at this time than is apparently the case in P. marinus unicolor, although Butcher does find that the original deutoplasmic material is absorbed rapidly in these cells.

The writer agrees that the appearance of the germ cells in these lampreys favors the theory of early segregation. While he finds himself unable to trace the germ cells in the present form as far back as has been done in E. lamottenii (wilderi) or in P. marinus, after the primordial sex cells are found near the epimere, they seem to be characteristically distinctive. The migration of these cells appears to be correlated with the progressive and determinative differentiation of the tissues along a metabolic gradient. Attempts by earlier investigators to assign an active or passive role to the germ cells during their translocation have not resulted in a satisfactory explanation to this date, in the opinion of this investigator; and much more research on the development of these sex cells is considered to be necessary before cumulatively established details will support some of the generalizations which have been made.

It does appear to be true that in later stages of development, the germ cells extend progressively along the longitudinal sagittal axis in both directions. However, unlike the circumstances reported for other brook lampreys, the germ cells in the present case do not always have a spherical shape, unless they are remarkably free from contact with adjacent cells. Okkelberg found in Entosphenus that some of the germ cells "remain in the endoderm" or in some other part of the body, and that they could form typical cell-nests there; this behavior has not been found in the local lamprey. As is well-known, although the original anlagen consist of paired germinal tracts, these fuse later to form a single longitudinal gonad ventral to the dorsal aorta. In connection with the development of this gonad, there has been found thus far no evidence in L. aepyptera of the Maryland race that the germ cells are derived from peritoneal cells at any time. It is further apparent in the Maryland form that when the germ cells are first certainly discerned, they are associated with mesodermal derivatives as the latter are usually defined in vertebrates.

This lack of participation in the formation of sex cells by the peritoneal epithelium is reported also by Okkelberg; but Lubosch ('04) and Butcher ('29) have found differently. Butcher states that in P. marinus unicolor the changes of peritoneal cells into germ cells can be found early, e. g., in larvae 7 to 10 mm. long. Quoting: "In these individuals numerous cells of the peritoneal epithelium with deeply staining nuclei are seen to be gradually changing from an oval to a spheroidal form." He thought further that in this species most of the sex cells probably originate from the peritoneal cells. Since no closely sequential histological studies have been published of this alleged phenomenon, nor cytochemical tests made to establish the progressive differentiation as described, this conclusion is difficult of acceptance.

No attempt has been made to count the number of germ cells existing in the early stages, although characteristic mitoses have been found from the time the larva is 20 mm. long until definitive histological differentiation between the sexes is established (about 35 - 37 mm. length). Mitoses in larvae destined to be females produce relatively few persistent oögonial cells in each cluster, and these contain some cells which are destined to a futile end, from the apparently residual moribund cells and cytolyzed remains observed in later stages of development.

During the indifferent stage of mitoses in the early germ cells it is reported in E. lamottenii (wilderi) that the nucleus has two deeply staining nucleoli; Butcher reports one and sometimes two in P. marinus unicolor. Further, Okkelberg finds that in the 27.5 mm. long larva the nucleus is associated with a distinct centrosphere. In the Maryland form this plurality of nucleoli is not manifest, although in early stages there are granular configurations found which might lead to the conclusion that a plasmasome exists in addition to a nucleolus. These appearances are considered to be fortuitous. The presumptive centrosome has been noted in favorable material as a light halo-like area about a centriole, but no other definitive organization of this area has been discerned.

Lubosch ('04) believed that there is a yolk nucleus which initiates the formation of the deutoplasm; but the writer agrees with Okkelberg that this does not seem to be the function of Lubosch's observed structure in larvae up to the 35 mm. length in any case, and for the Maryland form it is doubted that it serves this alleged function in any way whatsoever.

Pietschmann ('33) in reporting the work of others on the history of ovarian tissues, describes two architectural patterns which are said to have been found in L. planeri. In the one a follicular epithelium comes to surround each egg, and forms a theca. Over this there is developed a peritoneal epithelium; and in between the two layers there is a connective tissue stroma which is laced with blood vessels. In the second the eggs are not organized in thecae, are clumped and the condition is referred to as "diffuse". In the Maryland form of L. aepyptera no such diffuse condition has been found. Each successfully developing egg has surrounding it a concentric layer of follicle cells supported by a thin connective tissue, and the whole is overlaid by a thin peritoneal epithelium. There appear to be no exceptions to this.

At the time of the differentiation of definitive oögonia in the fall of the first year, it appears that most, if not all of the oögonia have been established; by actual count of secondary oöcytes just before spawning, the number surviving does not appear to exceed 1,200.

Okkelberg thought that the nucleoli were "true plasmasomes" and that they were not composed of chromatin material. This concept is contrary to that of Lubosch, who held that in the European brook lamprey the chromatin material is stored in the nucleolus (with particular reference to a structure in the primary oöcyte) and that the chromosomes were spewed out of the nucleolus to arrange themselves for the first meiotic division.

From such characterizations it will be observed that there is a real danger of misidentifying the nature of the nucleolus and of the germinal vesicle. The structure designated as the nucleolus in the oöcyte does not appear to be typically such, and because of this the writer was able to determine the nature of the nucleus only upon dissection in vivo. It is suspected that the so-called nucleolus partakes of the nature of a chromosomal frame as found in the Amphibia. At the time of the first meiotic metaphase a peculiar phenomenon is apparent, and which may have formed the basis for Lubosch's observation: when the chromosomes form the equatorial plate of the first meiotic division, a rest or shell-like structure is observed to one side in the nuclear field, and which has the dimensions generally of the so-called quondam nucleolus. It stains with iron-alum hematoxylin but disappears during the late anaphase of the first meiotic division.

Okkelberg found further that in _E_. _lamottenii_ (_wilderi_) the first maturation division occurs very early in development (the exact time is not specified); but in the present case this is not considered to be true. The first maturation division appears to take place in the Maryland form of _L_. _aepyptera_ at the same time, or at very nearly the same time, in both sexes: October or November of the third year.

In _L_. _aepyptera_ there has been found no evidence of the presence of any distinctively characterized sex chromosome in the oöcyte during the meioses. Only in the secondary spermatocyte is it believed that a sex chromosome (Y) has been observed in the present material, on the basis of its differential apparent rate of travel to one of the poles in meiosis. Accordingly it is presently believed to be true that in the local lamprey the XY configuration exists in the male chromosomal complement.

Okkelberg has found in Entosphenus that whether or not the germ glands become ovaries or testes, the latter all develop oöcytes, and he says that this indicates there exists an undoubted female element of some sort in all of the animals. In the present case the sampling made (about 425 animals) has not permitted the writer to come to any such conclusion. In E. lamottenii (wilderi) the basis for considering a larva male or female was the relative number of lobules (testicular stroma) or oöcytes present in the gonad; and it was held that if there is a majority of lobules containing spermatogonia, that the animal would probably become a male. Lubosch also found that hermaphroditism is of common occurrence in the larvae of lampreys. He examined forty-nine gonads from larvae of L. planeri, and found that about one quarter of them were "mixed glands". Nevertheless in these species the ultimate outcome was a very nearly equal frequency distribution between the two sexes. It is not denied that such hermaphroditism may exist in the present case; however the experience of the present investigator has been that the specific sexual characteristics are established at fertilization, and the ambivalence credited to other species appears to be peculiar to them. Such hermaphroditism has not been found in the present case.

The embryogeny in brook and riverine lampreys as reported, chiefly for L. fluviatilis, L. planeri and P. marinus, appears to follow a common basic pattern, but differences become manifest in the details of developmental progress when one species is compared to another. Also, there have been disagreements as to interprepation of these variations in detail, some of which still need resolution.

The most thoroughgoing account of early embryogeny in the genus Lampetra has been furnished by Glaesner ('10, '10a) who described the early development in L. fluviatilis. This was accompanied by a somewhat mechanistic interpretation of the earlier developmental stages. Weissenberg ('26, '32, '34) was able to reinterpret some of these stages of development through methods of vital staining, and he established satisfactorily the mode of gastrulation and the orientation of the germ layers in L. fluviatilis. By and large, the process of cleavage, blastula formation and gastrulation in L. aepyptera of the Maryland race is not different from that hitherto described.

The appearance of the male pronucleus of this Maryland brook lamprey in its progress along the copulatory path parallels the condition as found in L. fluviatilis in that it is an approximately ovoid body, but with uneven protuberances appearing from time to time in its periphery. The Chromatinbrocken of Glaesner which appear in L. fluviatilis as the pronuclei come to conjugate position, apparently are the chromosomes which will be seen in the metaphase plate of the first cleavage mitosis.

In L. aepyptera the more hyaline cytoplasm, at the time the pronuclei meet, is not constricted from the superficial polar plasm in so characteristic a fashion as appears to obtain in L. fluviatilis. After the first cleavage division is accomplished, however, the migratory behavior of the nuclei appears to be the same in both instances; they move approximately one third of the total distance from the animal pole. Glaesner found that at about 11° C. the time taken to accomplish the first division is about 18 hours or more. In the present case spawning appears to be stimulated when the waters of local runs are somewhat warmer (to 16° C.), although it is not doubted that it may occur at lower temperatures. It does take the Maryland brook lamprey less than 18 hours to accomplish the first cleavage division at these temperatures.

It is reported also in *L. fluviatilis* that the plane of the second blastomeric division is continuous throughout the embryo from the beginning of the cleavage, and that a shifting of the four resultant blastomeres takes place subsequently to establish the characteristic offset position which appears at the completion of the four-celled stage. In *L. aepyptera* of the Maryland race there is some variability in this pattern, including the fact that one of the first cleavage blastomeres may accomplish division before the other. This has happened with sufficient frequency to assure that the planes of the second division in the Maryland lamprey are the resultants of the mitotic behavior of the individual blastomeres. There is, therefore, an independence of kinetic behavior of the individual blastomeres, modified only in that both second cleavages have been found to occur in the same direction. Usually the offset pattern develops from the very beginning of the second division; but the distance of the offset is variable, and sometimes the cleavage planes appear to coincide. The writer is persuaded, however, that this is circumstantial. It should be stated that in the Maryland brook lamprey the second cleavage is not invariably meridional; on rare occasions it appears to be horizontal. This was observed in two instances, one of which was permitted to go to third cleavage; this was meridional, restoring the ordinary octuplet condition. When the second division is horizontal, it takes place above the equator, thus producing two micromeres and two macromeres.

A contributing factor to the production of division planes in other than the usual pattern is the accidental or deliberate canting of the egg in its brook bed after the two pronuclei have approximated one another incidental to the first cleavage, or during other early cleavage stages. Unlike the amphibian egg the embryo of the Maryland brook lamprey does not rotate in its envelope to maintain the position of the animal and vegetal pole. Apparently geotropic factors operate upon such canted eggs and cause shifts in the distribution of cytoplasmic materials with concomitant changes in potential organization, and anomalies in cleavage and further development result. They are productive of short-lived monsters.

Discounting these exceptions, the writer finds that there is a recognizable regularity in the division patterns up through the fifth generation of cleavages, although it has not been possible yet to evaluate the potentialities of the individual cells in successive generations. The methods of enumerating cleavage cells as employed by Glaesner appears to be purely a geographic device, and useful only in that respect.

It is apparent to the writer that in the developing embryo of L. aepyptera the fertilized egg and its developmental stages are highly organized, complex structures. The distribution of cytoplasmic materials at the first mitotic division shows patterns that await further interpretation, and to which the potentiation of succeeding generations may be referable. The arrangement of the cells in the cleavage and blastula stages seems to show considerable uniformity from embryo to embryo. As a consequence, Glaesner's opinion that in the blastula, for example, "Die Zellteilungen treten ohne bestimmte Regel bald hier, bald dort hin" appears not to be well grounded. The obvious determination and orientation of anlagen of the germ layers and then progressively of organs and tissues is considered also to support this writer's point of view.

The diagrams of the lampetrid blastulae hitherto published, notably for L. fluviatilis (Glaesner) show a maximum of three layers of micromeres, and the macromeric arrangement appears to show more, although stratification is less marked here. These findings are at some variance with the arrangement found in L. aepyptera; there appears to be a maximum of four layers in the micromeric hemisphere, and a fairly stratified triple layer of macromeres in the vegetal hemisphere.

The bilateral symmetry which has been described as existing in L. fluviatilis just before the time when the micromeric hemisphere initiates epiboly, may indeed exist in the present case, but there has been found no convincing evidence to the present time. There is indeed, a shift in the axis of the metabolic gradient in the Maryland brook lamprey, but this follows the epiboly down what becomes the dorsal side, thus indicating that the symmetry is progressively established during this event. The central point of the most rapid division migrates, as in other brook lampreys, towards the dorsal lip of the blastopore.

The movements of the micromeres and macromeres in the Maryland brook lamprey appear to create slightly different patterns in the germinal layers during the course of gastrulation from those hitherto reported, although the end result of the development appears to be the same as that reported in both the European brook and riverine species. Both Glaesner and Weissenberg indicate that the blastocoelic cavity in L. fluviatilis but slowly fills up during the early stages of gastrulation. The macromeres fill this cavity much more speedily in the present case, and at the time the primitive gut begins to progress inward, the blastocoel is only a slim, uniformly spheroidal cleft throughout. Likewise there is a definitely organized arrangement of the macromeres (endoderm) throughout, which Glaesner neither portrays nor implies. While the flange produced by the micromeres in their epiboly over the macromeres is reported as a strikingly definite structure in L. fluviatilis,yet, curiously enough, Glaesner could also say "Auch kann ich nicht mit Bestimmtheit behaupten, dass sie regelmässig auftritt". Furthermore, the protuberances of the torula stage do not seem to derive from the "vorragenden Enden der Leiste" (the projecting edges of the epibolic flange) as Glaesner thought; in the present case the frontier of the micromeres produces no perceptible flange for this purpose.

Glaesner held that the protuberances of the torula arise from the confluence and resultant pressures of the rapidly dividing cells in the area of the dorsal lip of the blastopore. It may well be. However in this Maryland brook lamprey the association of cells in these places appears to be also a specific response to stimuli arising in the course of organizational development, and the confluence of cells, if such it is, seems to arise from a complex of forces which are yet to be analyzed. It is projected that by this time in L. fluviatilis the center of the greatest metabolic activity has now centered at a point on the dorsal side approximately midway between the mid-dorsal point and the dorsal lip of the blastopore. In the present case this area is not so isolated as diagrammed by Glaesner; it is band-shaped in the Maryland brook lamprey, and lies athwart a plane drawn through the original line of the poles. This area consists eventually of two components,one of which comes to lie at the dorsal and lateral lips of the blastopore, and a central dorsal area which is to participate in the development of the medullary plate.

As has been noted, the development of the archenteron in L. fluviatilis occurs when the blastocoel is still a considerable cavity, and the primitive gut soon modifies the internal macromeric floor. In L. aepyptera there appears to be no extensive blastocoelic cavity at the time that invagination is initiated; there is a high order of histological differentiation in the several parts of the embryo; and the shape of the archenteron is different. One circumstance rather frequently noted in the present case is that at the time of the intussusception of the micromeres, the high dome of the micromeres in imbedded material may collapse to one side during the infiltration incident to this process, and the overhanging ledge so formed, when sectioned, can have somewhat the same appearance as diagrams of gastrulation hitherto depicted. In fact, this artefact may be very subtle in its appearance.

It remains to be noted that Weissenberg, through his vital staining experiments came to the conclusion that the mesodermal anlagen initially forms the dorsal roof of the archenteron, and that the endoderm overreached this cavity dorsally by growth from the sides. With this finding the present writer is in accord with respect to the present species, and is undertaking further research to determine the particulars in *L. aepyptera* of the Maryland race.

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