CANADIAN SPECIAL PUBLICATION OF FISHERIES AND AQUATIC SCIENCES 49

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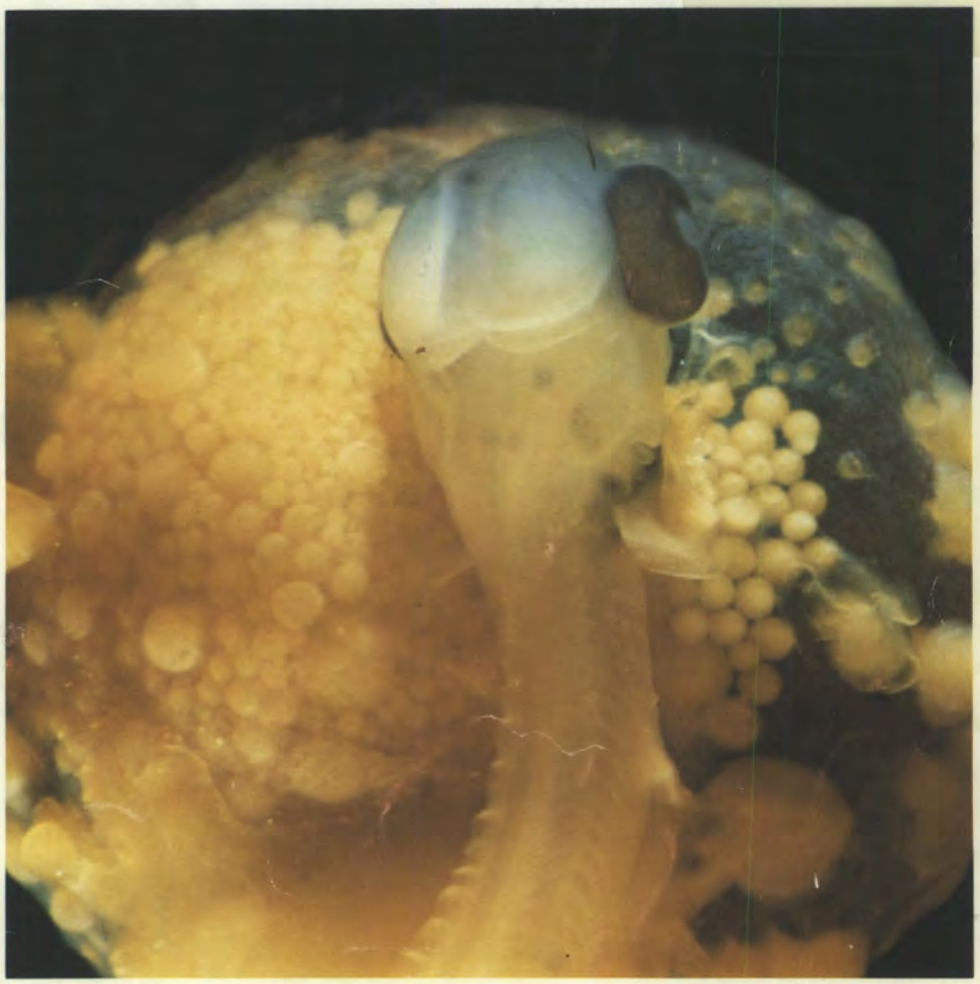

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Embryonic Development in Eggs of Sockeye Salmon, *Oncorhynchus nerka*

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DEPARTMENT OF FISHERIES AND OCEANS
Ottawa 1980

Published by

Government of Canada
Fisheries and Oceans

Scientific Information
and Publications Branch

Ottawa K1A 0E6

Publié par

Gouvernement du Canada
Pêches et Océans

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Canada:	$2.95	Catalog No. FS41-31/49E
Other countries:	$3.55	ISBN 0-660-10684-1
		ISSN 0706-6481

Price subject to change without notice

Printed in Canada

Correct citation for this publication:
VELSEN F.P.J. 1980. Embryonic development in eggs of sockeye salmon,
Oncorhynchus nerka. Can. Spec. Publ. Fish. Aquat. Sci. 49: 19 p.

Contents

Abstract

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Fisheries workers at hatcheries, spawning channels, and incubation box facilities may wish to identify stages of embryonic development reached by incubating eggs, or by eggs that died during development. Information on embryonic development and differentiation in Pacific salmon eggs in relation to water temperature is not readily found. This publication illustrates 20 easily identifiable stages of embryonic development in eggs of sockeye salmon *(Oncorhynchus nerka)*, and provides information on their rates of development at temperatures between 5 and 11°C. Details of differentiation and rate of development may vary somewhat among closely related fishes. Nevertheless, the stages illustrated for sockeye salmon should provide reasonable approximations to those found during egg development in all Pacific salmon.

Key words: Oncorhynchus, sockeye, embryology, eggs, incubation, temperature

Résumé

VELSEN, F. P. J. 1980. Embryonic development in eggs of sockeye salmon, *Oncorhynchus nerka*. Can. Spec. Publ. Fish. Aquat. Sci. 49: 19 p.

Le personnel chargé du fonctionnement des établissements de pisciculture, chenaux de ponte et installations de boîtes d'incubation désireront parfois identifier les stades de développement embryonnaire d'oeufs en voie d'incubation, ou d'oeufs morts au cours du développement. Il n'est pas toujours facile de trouver des renseignements sur le développement embryonnaire et la différenciation des oeufs de saumons du Pacifique, en relation avec la température de l'eau. Nous illustrons dans la présente publication 20 stades facilement identifiables de développement de l'embryon dans l'oeuf de saumon nerka *(Oncorhynchus nerka)* et présentons des données sur leur taux de développement à des températures de 5 à 11°C. La différenciation et le taux de développement peuvent varier quelque peu dans le détail entre espèces étroitement apparentées. Néanmoins, les stades applicables au saumon nerka devraient donner une assez bonne idée de ceux observés au cours du développement des oeufs de tous les saumons du Pacifique.

Introduction

Salmon are cold-blooded animals, or poikilotherms; their body temperature remains close to that of the water in which they live. This association between the temperature of the animal and that of its environment has a dominant influence on the life of the salmon. Most of the salmon's life processes occur more rapidly at higher temperatures, including ability to swim, the amount it eats, how fast it grows, and the rate at which its eggs develop once they are deposited.

Like the other four species of Pacific salmon found in British Columbia, the sockeye salmon *(Oncorhynchus nerka)* returns to fresh water to spawn. Sockeye spawn in rivers, streams, and lakes in the autumn when water temperatures are declining from the summer maxima. Spawning adults may lay their eggs in the gravel of streams at temperatures up to 12°C, and occasionally higher. At these temperatures, initial stages of egg development occur rather rapidly. However, as autumn advances and water temperatures continue to decline, the rate of egg development slows down accordingly. Development is slowest when winter minimum temperatures are reached from near zero to about 2°C. Between December and February the eggs hatch; the actual time depends on preceding temperatures to which the eggs were exposed. The newly hatched salmon larvae, or alevins, are embryonic fish with a large yolk sac. The alevins remain in the gravel, using their yolk supply for continued growth and development. When water temperatures begin to rise in early spring, the alevins will have used most of their yolk. Not long after, usually in April or May, the alevins work their way to the surface of the gravel to emerge as free-swimming fry.

In British Columbia, a number of hatcheries, spawning channels, and incubation boxes are planned or are in operation to increase salmon runs. When eggs from these facilities are sampled, questions may arise concerning their state of development. As the speed of development depends on temperature, the time required for an egg to reach a particular stage of development will depend on the temperatures experienced earlier. Conversely, if some eggs are dead and the previous temperatures to which the eggs were exposed are known, then the stage of development seen in the dead egg will provide an estimate of when each egg died. For example, if the average incubation temperature has been 7°C for a period of 20 days since fertilization, the eggs would be expected to be near Stage 16 (Table 1). The basis for this time-temperature relation may be expressed approximately in degree-days[1] to indicate the state of development expected after a given incubation period at known temperatures. Information of this type is surprisingly scarce for Pacific salmon; normal rates of embryonic development are largely unknown. This publication illustrates some easily recognized stages of development from fertilization until hatching.

The eggs were obtained at the Fulton River, Babine Lake, British Columbia. To obtain examples of the various stages of embryonic development with known temperature histories, the eggs were fertilized in the laboratory and cultured at three constant temperatures of 5, 8, and 11°C. Eggs were sampled throughout the incubation period and preserved in (a) 5% neutral formalin[2] for internal examination after removal of the shell or capsule, and (b) in Stockard's solution[3] for external examination through the partly transparent shell.

From fertilization to a time just prior to hatching the egg capsule is translucent white and the orange-colored yolk gives the egg its characteristic color. Just prior to hatching the capsule develops an opalescent sheen, presumably a result of the hatching enzyme acting on the inner surface of the capsule. For these reasons special techniques must be used throughout egg incubation so that embryonic development in salmon eggs can be observed. Hence, many workers will not have had the opportunity to see most of the developmental stages, such as those illustrated here. The use of Stockard's solution makes it possible to see some of these stages without opening the egg. The internal structures of dead eggs in water turn white and become opaque; such eggs may be "cleared" in Stockard's solution to reveal internal details that otherwise could not be seen. Preservation in neutral formalin hardens the yolk and delicate embryonic tissues; after about 2 wk such eggs may be opened with minimum risk of damage to the delicate embryonic structures.

Thirty stages of development can be recognized in the sockeye egg, from fertilization to hatching, as adapted from Vernier's (1969) description of embryonic development in the rainbow trout, a similar salmonid fish. Figures 1–20 illustrate 20 readily identifiable stages with the shell removed. Nine of these stages are shown, for comparison, as photographed through the egg shell (Fig. 3b, 4b, 5b, 12b, 13b, 14b, 16b, 17, 20b). Next, the time in days for eggs to reach various stages of development, from Stage 2 through Stage 30, and the time of 50% hatch, is shown (Fig. 21) for the three constant incubation temperatures (5, 8, 11°C). Between the three test temperatures, similar relations are shown by interpolation at intervening temperatures of 6, 7, 9, and 10°C. Finally, the actual and interpolated development times used to construct the graph (Fig. 21) are listed in Table 1.

[1] One degree-day is the mean temperature, above 0°C, experienced for a period of 24 h. A salmon egg incubated at an average daily temperature of 10°C for 62 days, from fertilization to hatching, is said to have hatched in 620 degree-days.

[2] 50 mL formaldehyde, 4.0 g sodium phosphate monobasic, 6.5 g sodium phosphate dibasic, made up to 1 L with distilled water.

[3] 50 mL formaldehyde, 40 mL glacial acetic acid, 60 mL glycerin, and 850 mL distilled water.

Description of Developmental Stages

The egg is fertilized when one spermatozoon from the milt of the male finds and enters the micropyle, a narrow canal in the capsule. Thereafter, other spermatozoa are prevented from entering the micropyle by a series of events triggered by the entry of the first sperm. All eggs, fertilized and unfertilized, react to the water in which they are deposited. This action involves a swelling and increase in internal pressure (hardening), as water is taken up by the egg. The embryonic development that follows fertilization may be divided into three phases — cleavage, gastrulation, and organogenesis (Table 1). These phases are described in the following sections.

Cleavage

After fertilization, a colorless cell fluid, the cytoplasm, begins to migrate over and concentrate in one location on the surface of the yolk — at the animal pole. There the cytoplasm rounds up and rises slightly to form a hemispherical dome. This initial cell of the blastodisc (Stage 1, Fig. 1), is the cell from which the embryo will form by cell division and differentiation. Underlying the disc, visible in the figure, is a group of fine oil globules. These become opaque yellow in the formalin preservative, distinguishing them from the white color of the preserved blastodisc. This organization of the cytoplasm appears to occur both in fertilized and unfertilized eggs (Soin 1953). The blastodisc remains for a time in the unfertilized egg, then begins to collapse and deteriorate as time progresses. It continues to develop in the fertilized egg. Later, when the eyes are forming in the embryos of developing eggs, there is usually no remaining evidence that a blastodisc was present in the unfertilized egg (Fig. 2).

In the fertilized egg, cell division begins with the first cleavage of the blastodisc. It divides to form two cells (Stage 2, Fig. 3a, b), and each of the two new cells divide to form four (Stage 3, Fig. 4a, b). By successive divisions 8, 16, and 32 cells are formed (Stages 4, 5, 6; Fig. 5a, b, 6, 7). The initial cell divisions occur in the horizontal plane. After Stage 6 some divisions occur in the vertical plane and, therefore, succeeding stages cannot be easily identified by cell number. As cleavage continues, the resulting cells become smaller and smaller. After further cell divisions, the early and late morula stages are formed (Stage 7, 8; Fig. 8, 9). The morula begins to have a granular appearance; the individual cells may still be seen but become difficult to count. After the late morula stage the multicellular disc begins to thicken around the edges; the edges then subside and the disc flattens to form the blastula (Stage 10, Fig. 10). At this stage, the future location of the embryo, yet to form, can be detected on one side of the disc as a thickening of the edge. This ends the cleavage phase, characterized by the proliferation of cells.

Gastrulation

The second phase begins with the formation of the gastrula or early embryo (Stage 12, Fig. 11). During gastrulation the cells formed in the cleavage phase begin to specialize to form tissues, and it is during this phase that the embryo is formed (Stage 13, Fig. 12a, b). The edge of the blastodisc expands and grows down over the surface of the yolk (Fig. 12). The overgrowing edge, or germ ring, is preceded by a clear layer of cytoplasm called the periblast, or syncytium (not visible in photograph). The overgrowth of the yolk by the germ ring, part of a process called epiboly, continues. The tissues formed by the germ ring overgrowth eventually envelop the yolk in what is destined to become first the yolk sac and later the tissues enclosing the body cavity of the young fish. At the same time the organization of the embryo is becoming more apparent. The head of the developing embryo is at the original animal pole, and the rudiment of the tail is near the border of the germ ring. It is convenient to follow development at this time in terms of the extent of the germ ring overgrowth, or epiboly. By Stage 13, one-third epiboly has occurred (Fig. 12). By Stage 15 it is two-thirds complete (Fig. 13a, b) and by Stage 16, the germ ring is three-quarters overgrown. As epiboly nears completion, an area below the future tail remains open (Fig. 14a, b). This area, called the blastopore, will diminish in size, ultimately "closing" to become the vent, or anus, of the developing embryo. At one-third epiboly the head can be seen as well as a few somites, the embryonic precursors of muscle tissue in the trunk or body region. The optic vesicles, the developing eyes, may be detected as early as one-half epiboly; by two-thirds epiboly they have formed (Fig. 13b). The otic placodes, which develop into balance organs, are related to the semicircular canals of the inner ear of higher animals. The placodes (not visible in photograph) are near the first branchial pouch where the gill arches form. At blastopore closure (Stage 17) the yolk is completely enveloped by the expanding blastodisc, muscle somites are prominent in the trunk region, and a developing lens can be seen in the eye. The cells formed during cleavage now have developed into tissues in the gastrulation phase to produce the basic structure of the embryo.

Organogenesis

The third and final phase of development during egg incubation is characterized by the appearance of fins and formation of the internal organs and circulatory system. Immediately after blastopore closure the posterior end of the embryo extends to form the caudal bud, which lifts free of the surface of the yolk (Stage 18, Fig. 15). The caudal bud is the precursor of the caudal or tail fin of later stages. Observations of eggs preserved in Stockard's solution are limited now to stages 21, 22, and 24, those associated largely with yolk vascularization (the development of a network of

blood vessels covering the surface of the yolk from which food is supplied to the embryo.).

Yolk vascularization is an external indication of the developing circulatory system. By Stage 21 (Fig. 16a, b) the vitelline vein, emerging from the left side of the head region (Fig. 16b), encircles one-quarter of the yolk; the eyes now show faint pigmentation, which appears more concentrated around their perimeter (Fig. 16b). When three-quarters of the yolk surface has become vascularized (Stage 24, Fig. 17) the head is free, the eyes are fully pigmented, and the eggs are said to be "eyed."

The eyed stage is important in salmonid culture and requires some further comment. In salmon hatcheries, eggs generally are left undisturbed from the time they are fertilized until they reach the eyed stage. The pigmented eyes of Stage 24 are easily seen through the capsule of the live egg. From fertilization until closure of the blastopore (Stage 17) the egg is very susceptible to injury from mechanical shock. Simple movement of the egg in this period can damage the delicate embryo, which may develop abnormally and die later. In practical circumstances, however, the eggs are left until the eyed stage, when they have become highly resistant to mechanical shock. At this stage, the eggs in hatcheries are shocked deliberately. Any eggs that by chance were not fertilized, or died early in development, turn white after shocking and can be distinguished easily from the living eggs and removed. If an unfertilized and unshocked egg were removed at this time, preserved and opened, it would resemble that illustrated in Fig 2. Estimates of egg survival in salmon hatcheries commonly are made at this stage. However, one may wish to determine the success of fertilization much earlier than the eyed stage, as for example, when eggs are treated with antibiotics shortly after fertilization. Such eggs would be sampled and preserved after an interval sufficient for them to develop to stages 3 or 4. Any eggs that did not become fertilized then would resemble Fig. 1, a fertilized egg prior to cleavage.

Following the eyed stage (Stage 24), blood vessels continue to develop on the yolk. The head of the embryo rests on but is free of the yolk sac. For the next few stages the operculum, or gill cover, begins to grow to cover the branchial arches on each side of the head. The branchial arches will form the gills of the hatched fish. In Stage 25 (Fig. 18) the operculum has not yet covered the first branchial arch. By Stage 27 (Fig. 19) the first gill arch is covered. By Stage 30 (Fig. 20a, b) all arches are covered, all the paired fins are formed, and the pigment cells in the skin of the embryo give it a dark color. Externally, the embryo at this time appears completely formed and ready to hatch. However, the embryo remains within the capsule for a further period (Table 1, Fig. 21). Finally, the embryo secretes a hatching enzyme. The enzyme digests the capsule from the inside, weakening it until it ruptures to free the now very active embryo. Once hatched, the embryo with its large yolk sac is called an alevin. Hatching usually is rapid, once the capsule ruptures. However, all eggs do not hatch at one time, even though they have had identical temperature experience. In a hatchery tray of eggs incubated at one temperature, the eggs may hatch over a period of a few days or more. Also the hatching period is longer at lower temperatures.

In general, the stages of embryonic development and differentiation illustrated for the sockeye are quite similar in appearance to those of the other species of Pacific salmon. At a constant temperature of 10°C the incubation period to 50% hatch among eggs of the five British Columbia species ranges from about 47 to 65 days, that for the sockeye being the longest. Therefore, the development time required for eggs to reach particular embryonic stages, when held at a common temperature, would be somewhat shorter for the other species than for the sockeye.

Acknowledgments

The author gratefully acknowledges the assistance of Dr D. F. Alderdice for critically reviewing the manuscript, and for suggesting important additions and corrections.

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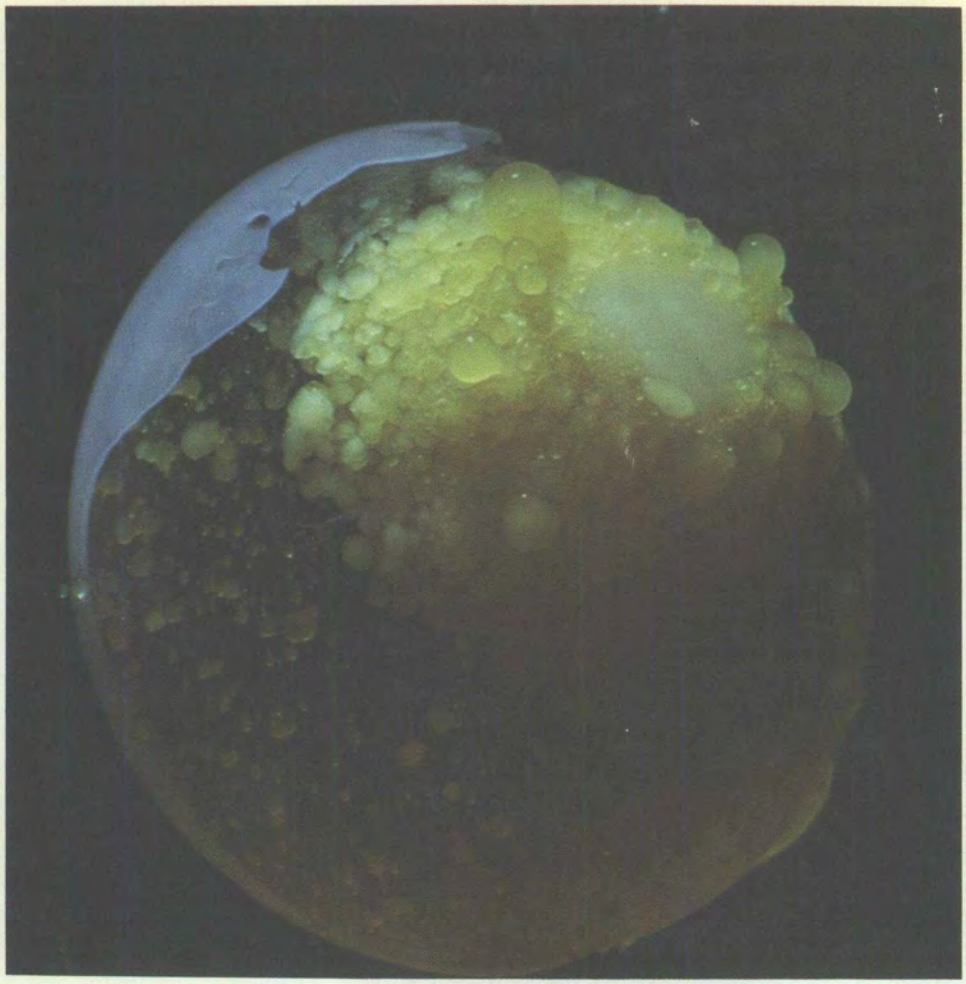


FIG. 1. (*left*) Fertilized egg. The cytoplasmic cap, or blastodisc, rests on a bed of oil droplets. Part of the egg capsule is visible between 9 and 12 o'clock. Magnification, ×19 (5% neutral formalin preservative).

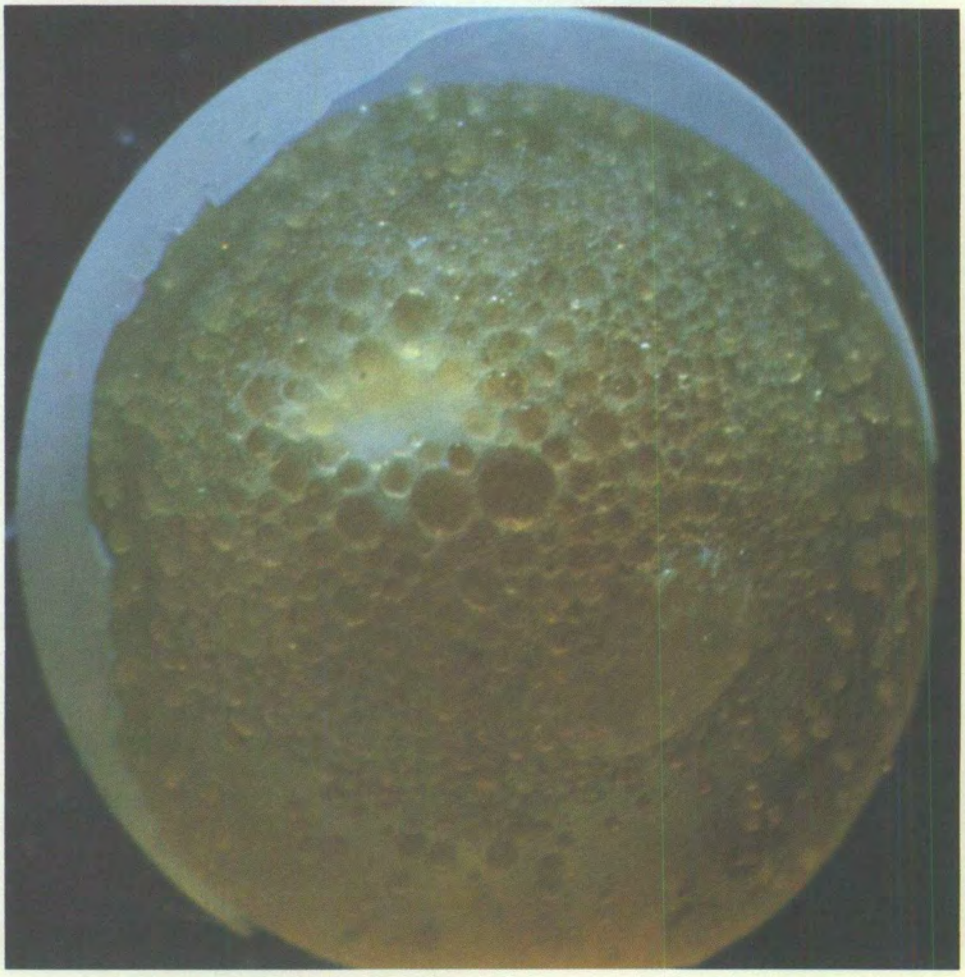


FIG. 2. (*right*) Unfertilized egg. Appearance of animal pole after 20 days at 10°C. The blastodisc has collapsed to form an irregular patch on the surface of the yolk. ×19 (formalin).

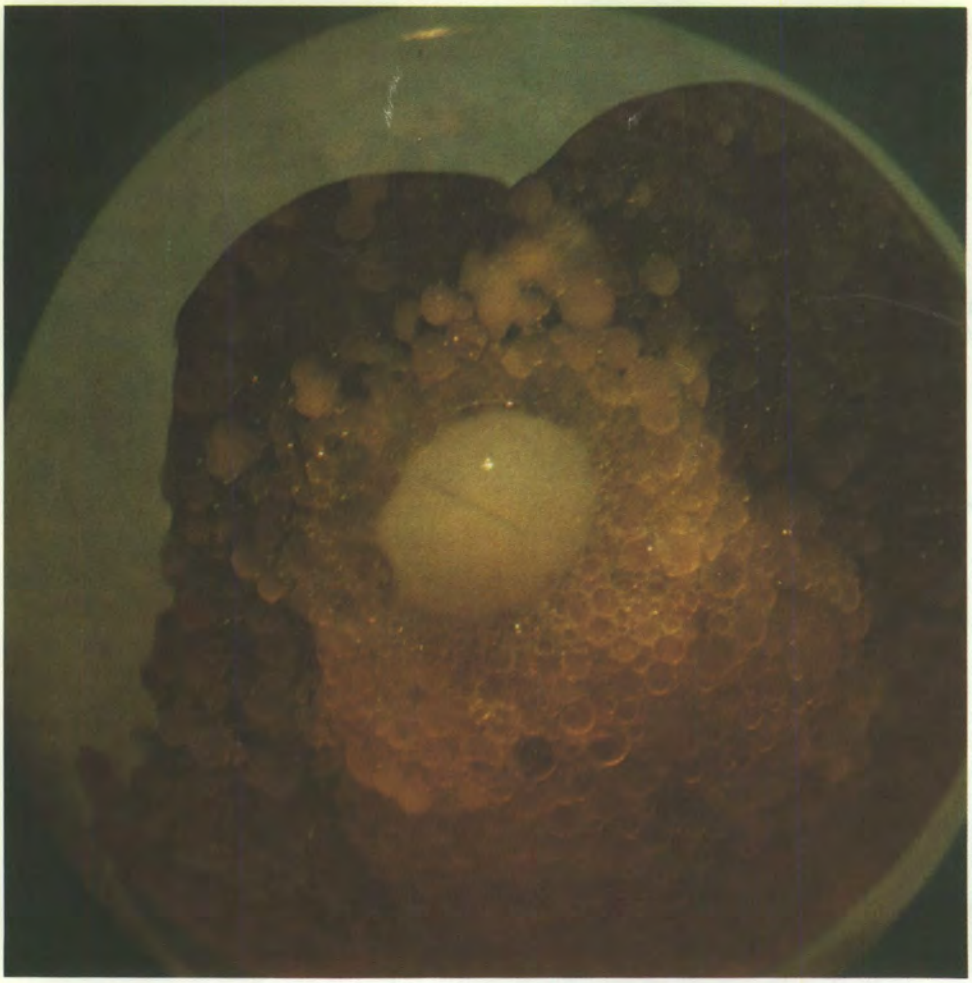
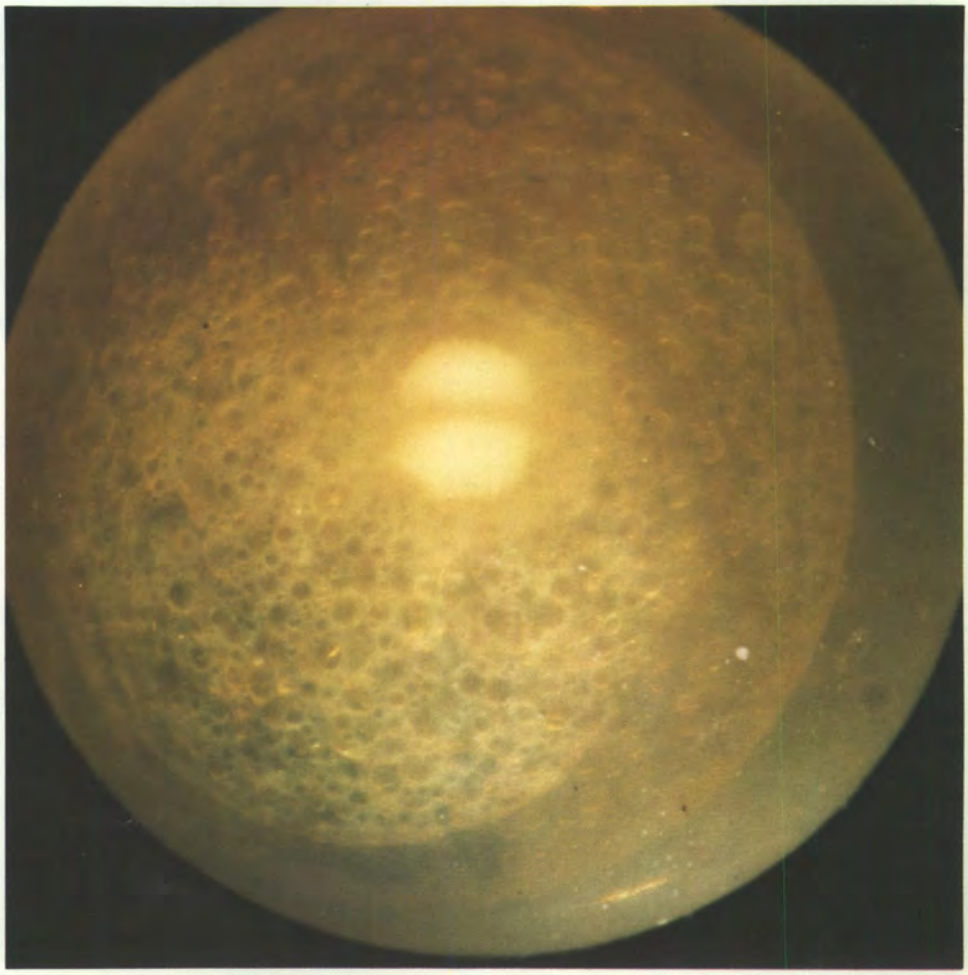


FIG. 3. Stage 2, two-cell stage after first cell division (a) (*left*) ×22 (formalin), (b) (*below*) ×21 (Stockard's solution).



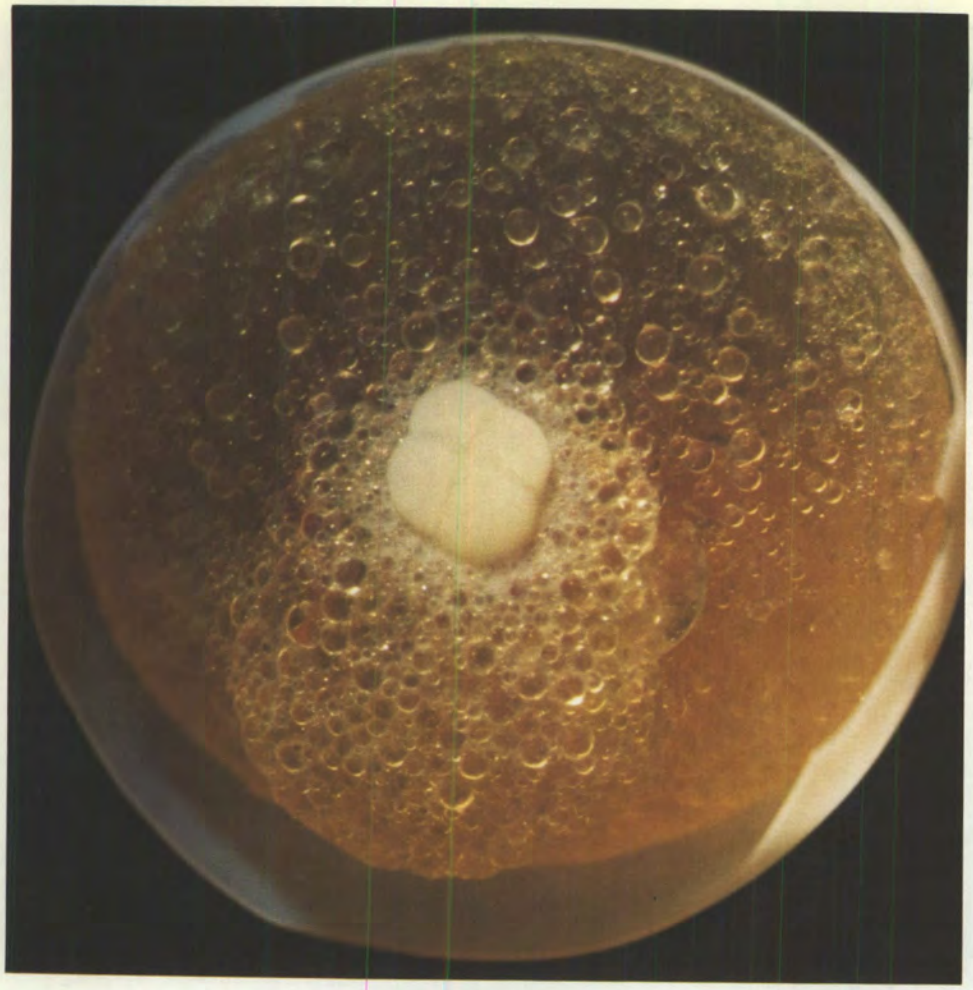
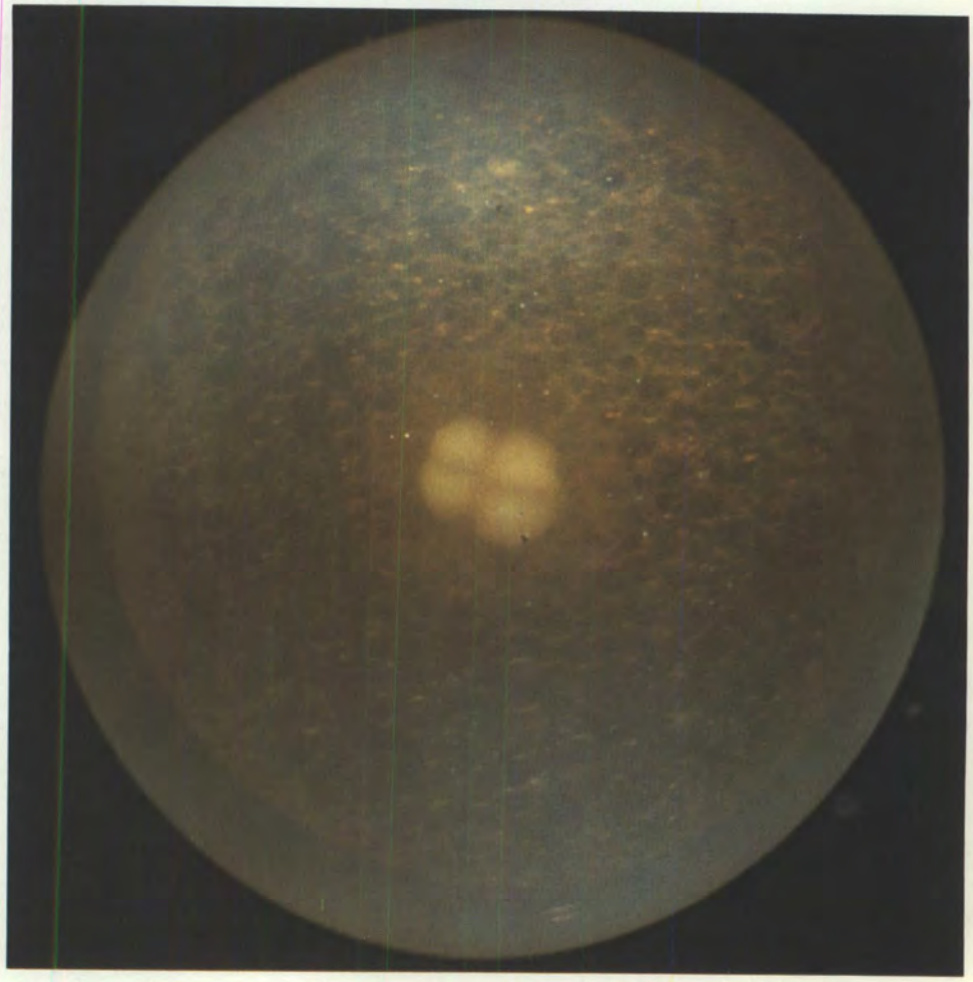


FIG. 4. Stage 3, four-cell stage (a) (*left*) ×19.5 (formalin, (b) (*below*) ×19 (Stockard's).



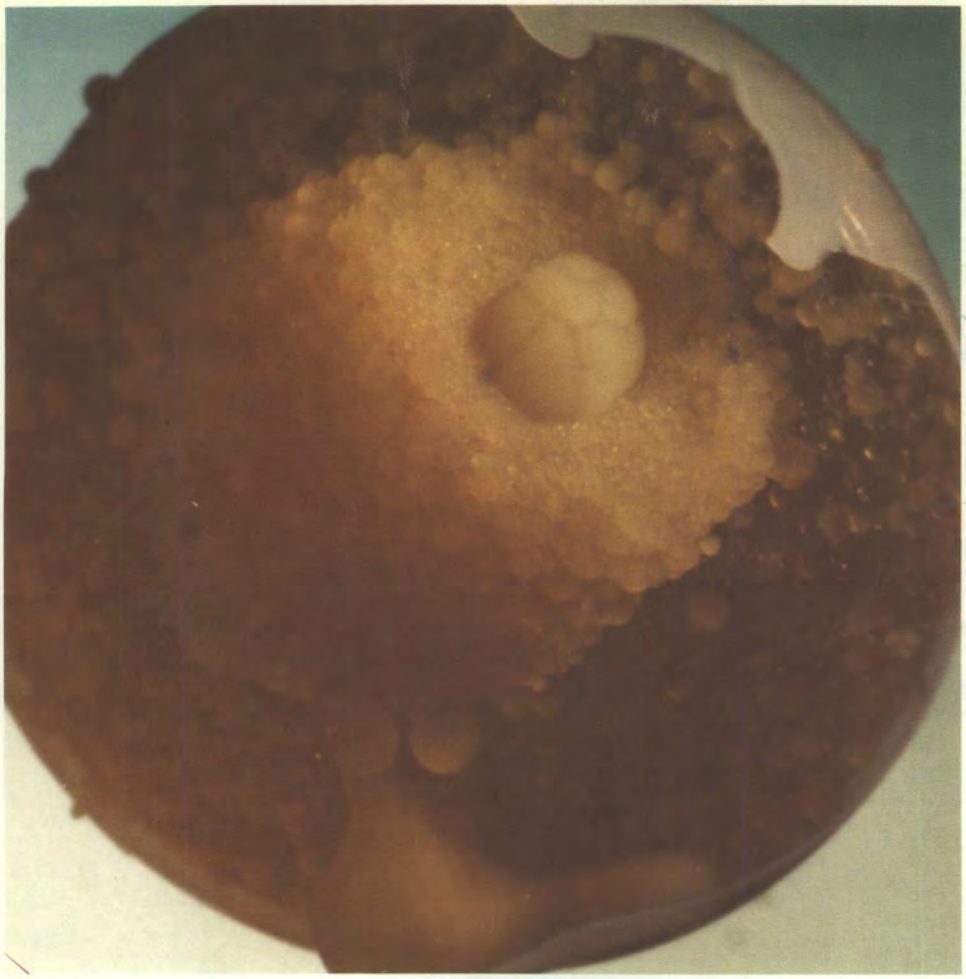
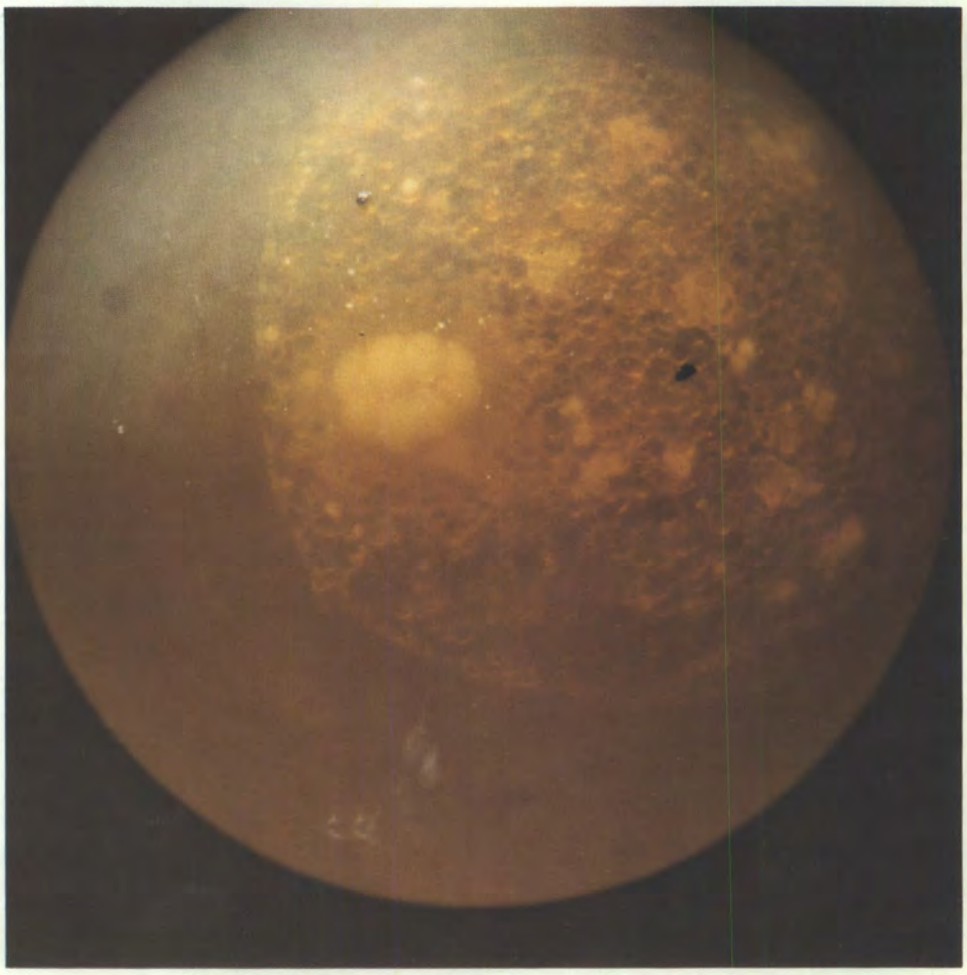


FIG. 5. Stage 4, eight-cell stage (a) (*left*) ×21.5 (formalin), (b) (*below*) ×21.5 (Stockard's).



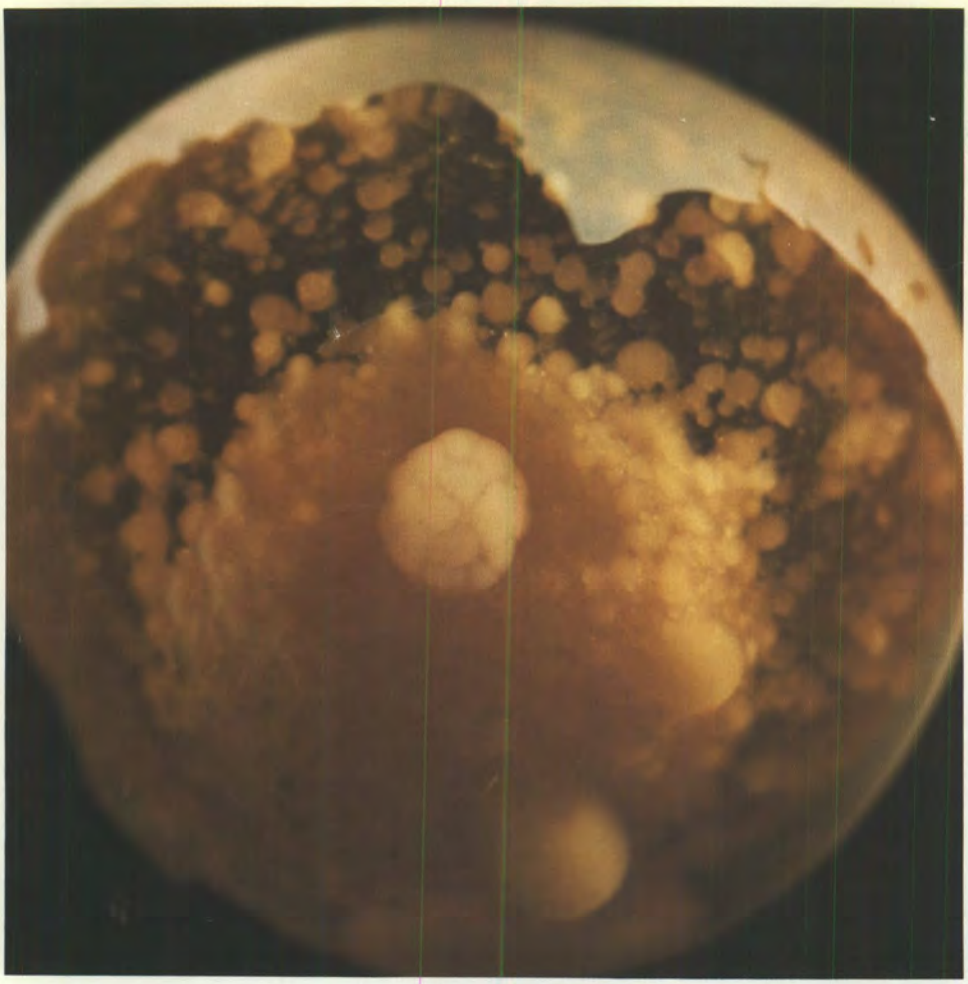


FIG. 6. (*left*) Stage 5, 16-cell stage. ×22 (formalin).

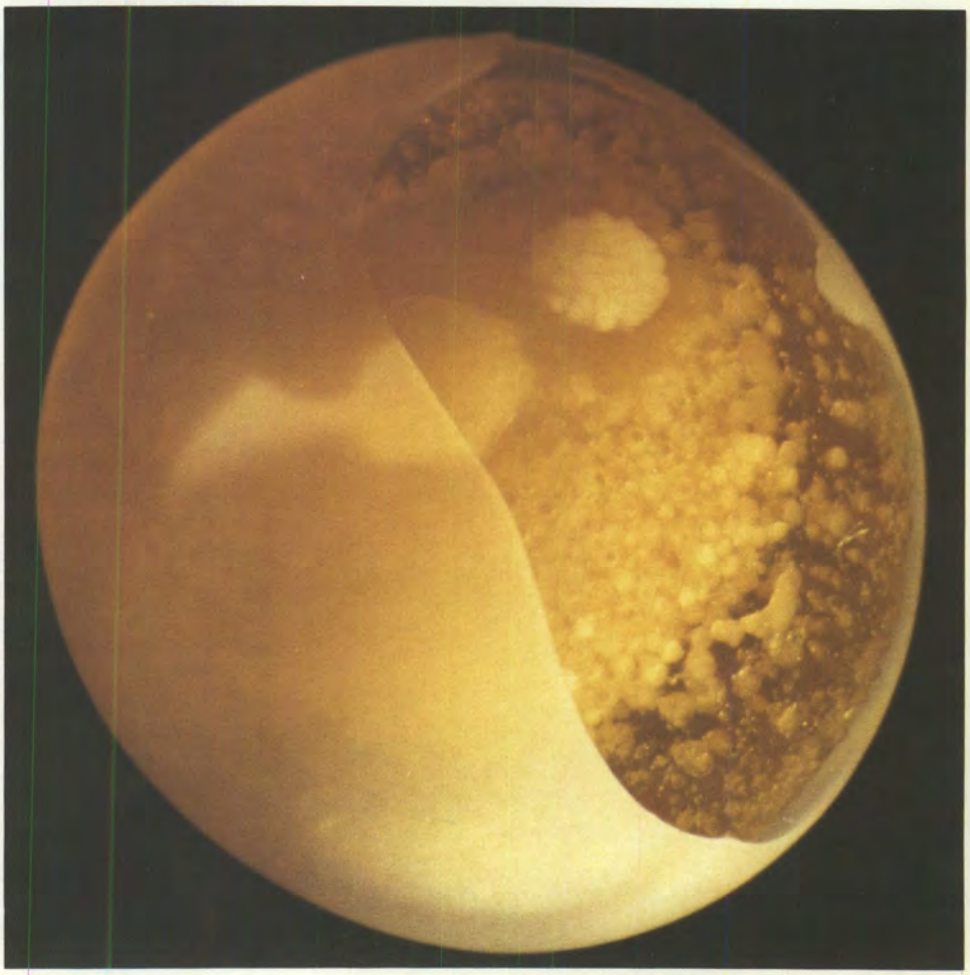


FIG. 7. (*right*) Stage 6, 32-cell stage. Cell division now continues in both horizontal and vertical planes. ×18.5 (formalin).

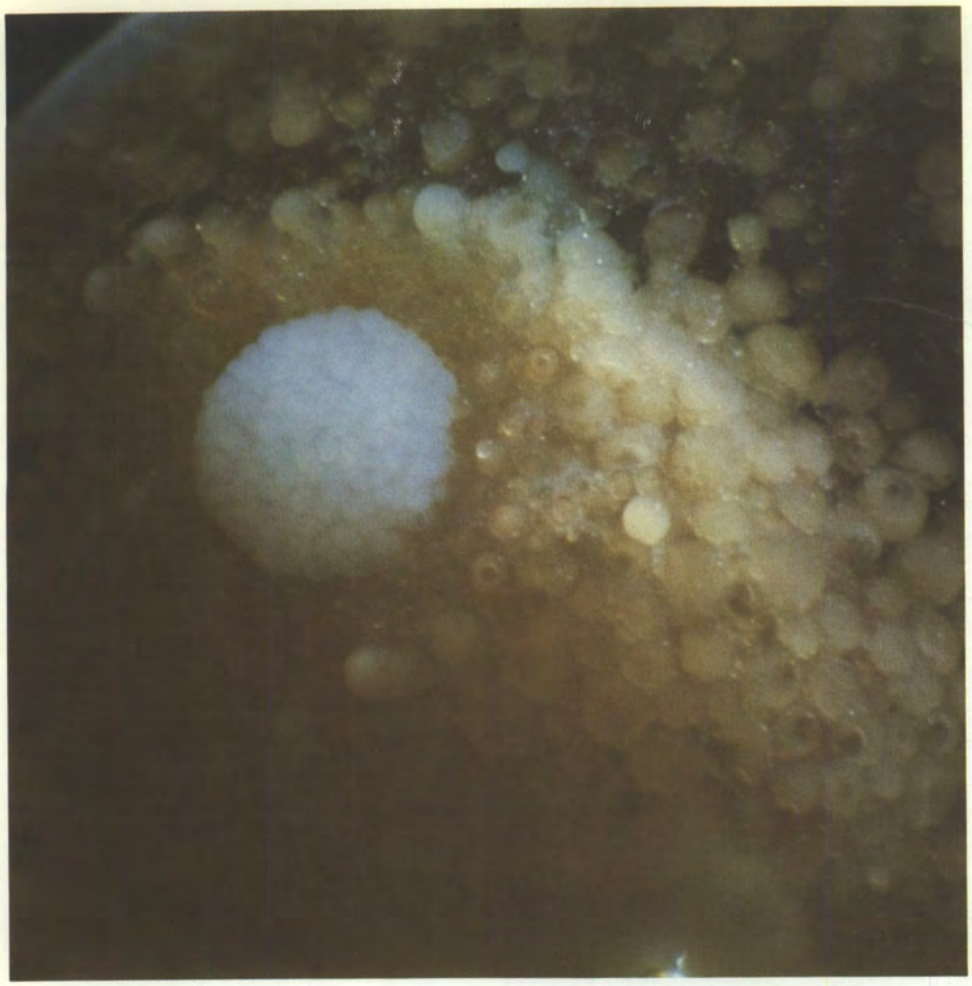


FIG. 8. (*left*) Stage 7, early morula. ×28 (formalin).

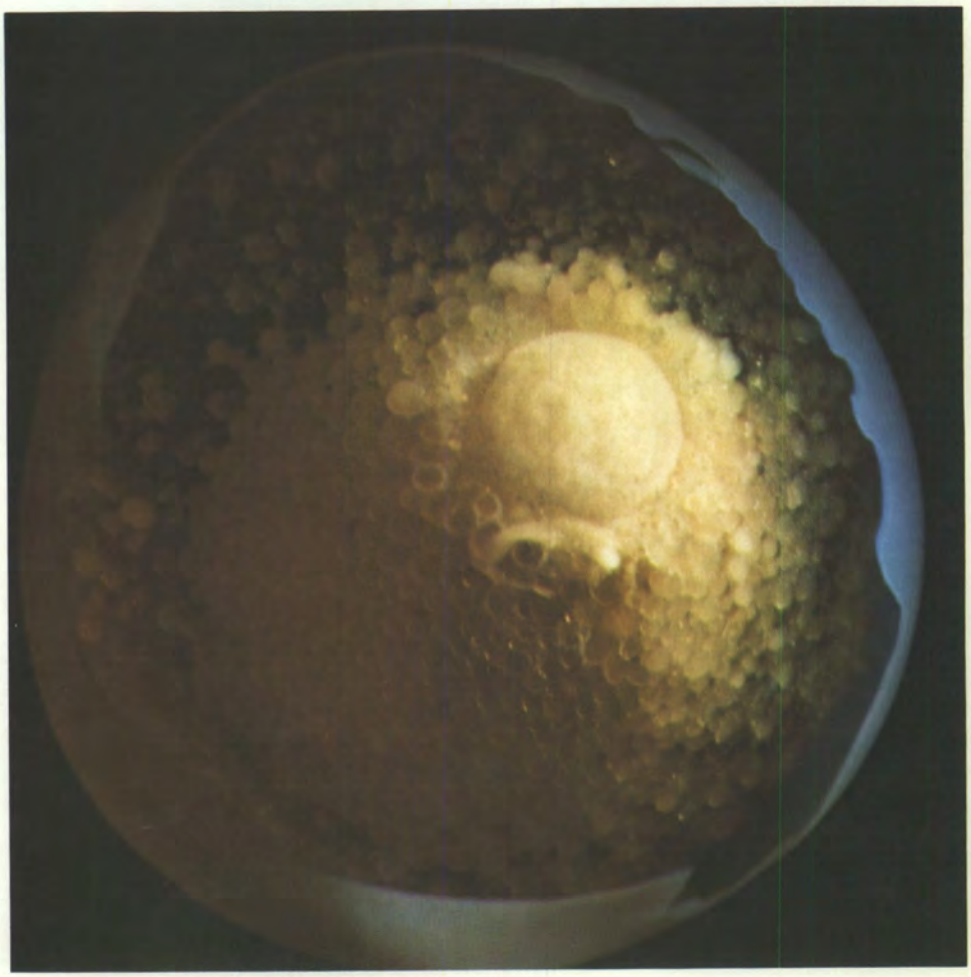


FIG. 9. (*right*) Stage 8, late morula. ×21 (formalin).

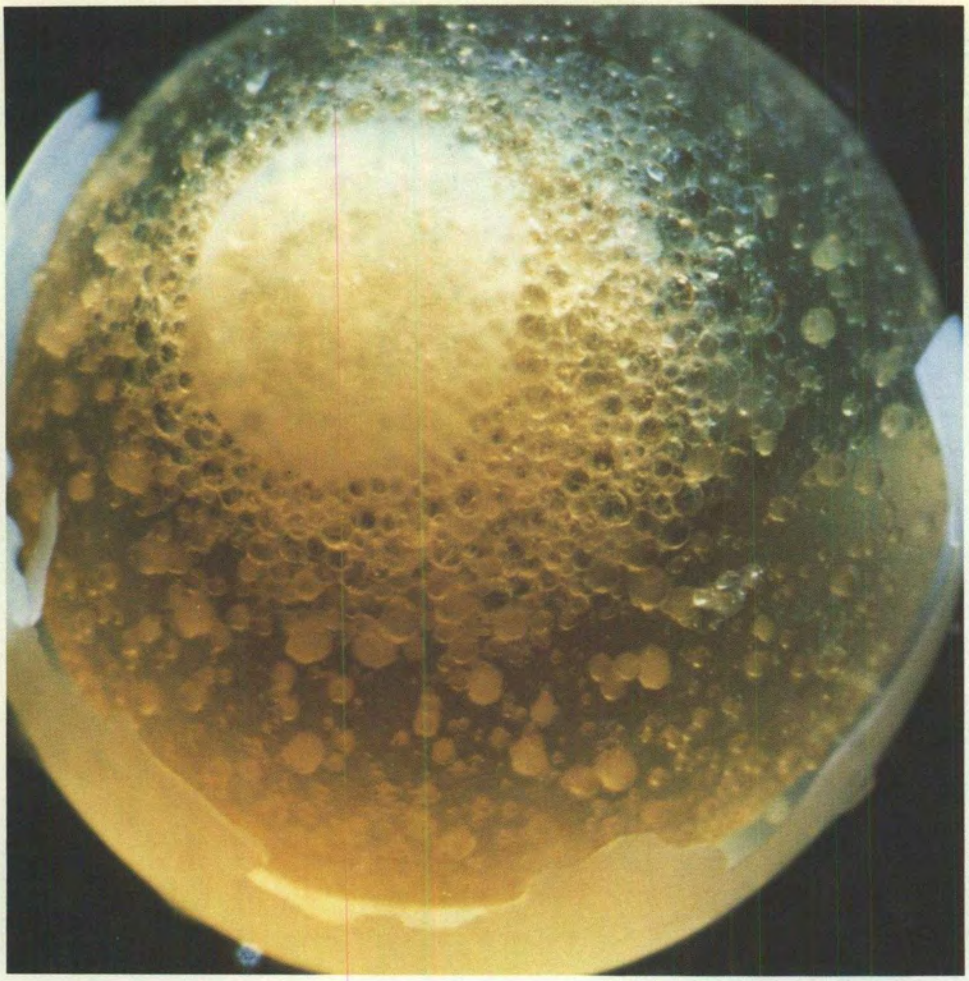


FIG. 10. (*left*) Stage 10, blastula. White rim indicates an increase in cells around the periphery of the disc. ×22 (formalin).

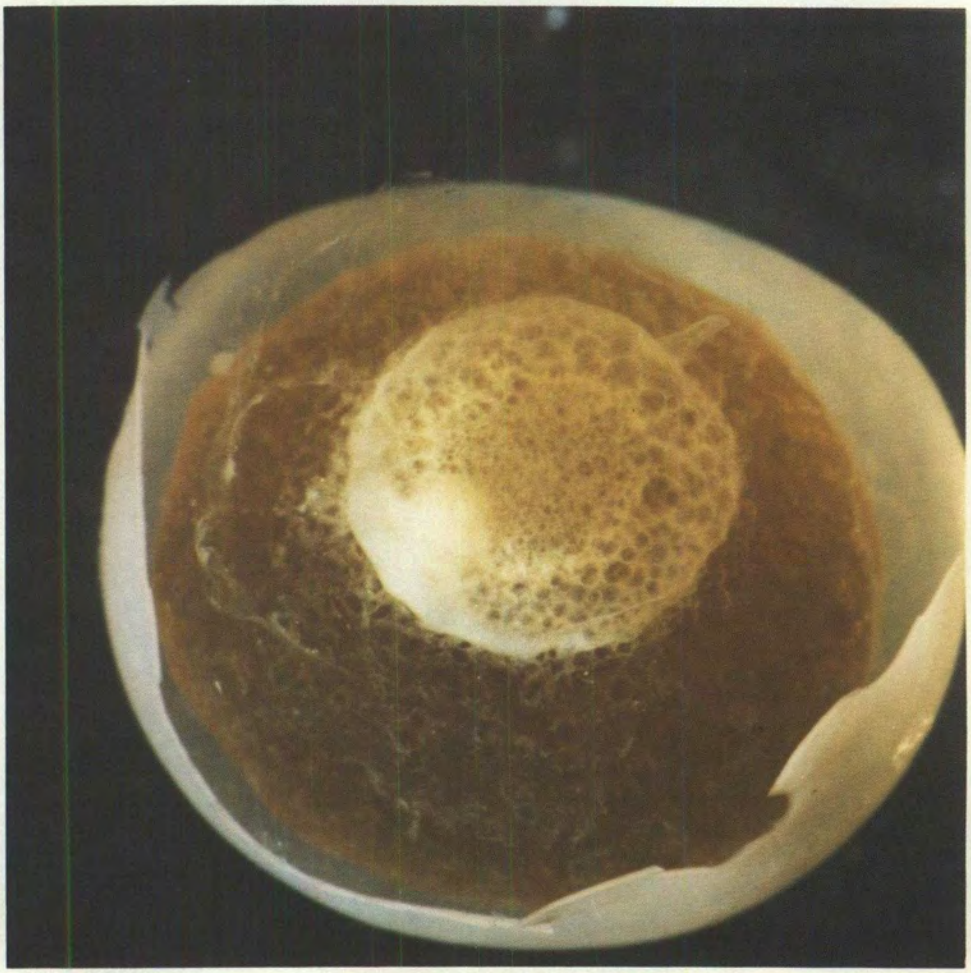
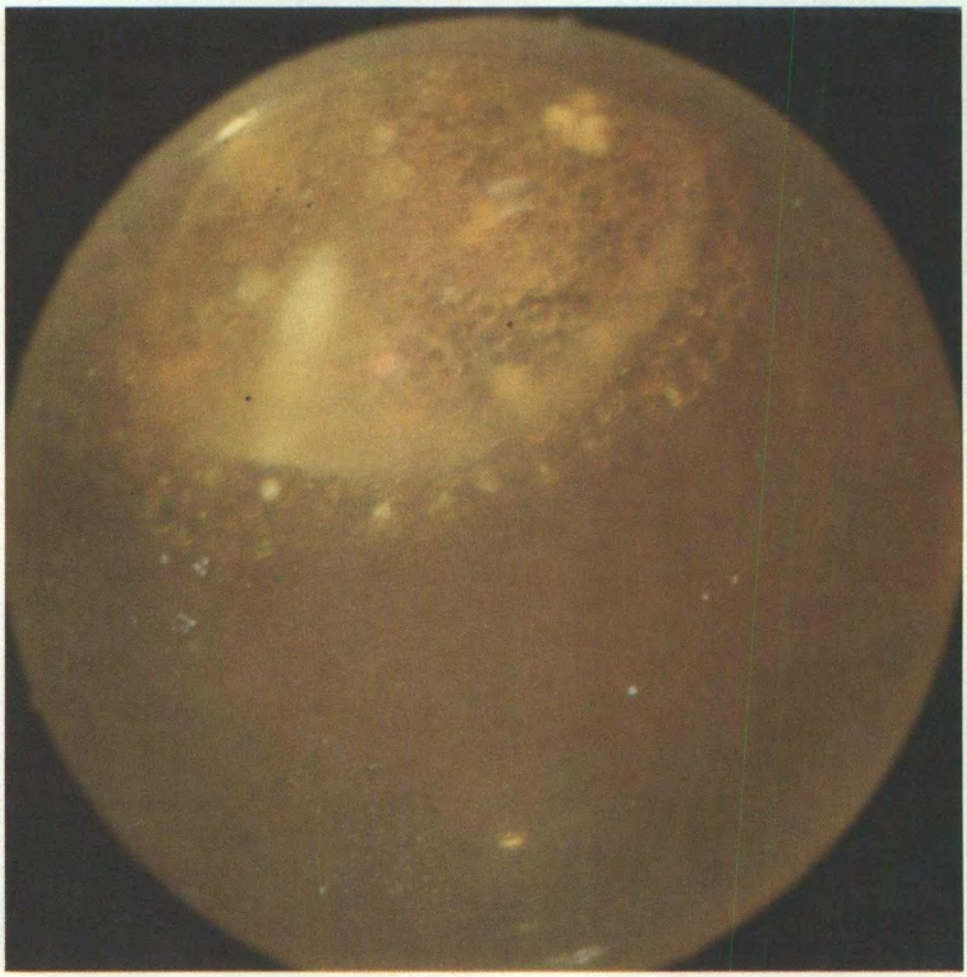


FIG. 11. (*right*) Stage 12, gastrula; formation of embryonic axis. Increasing cell concentration on left side of disc (about 8 o'clock) shows start of formation of embryo. ×18 (formalin).

FIG. 12. Stage 13, beginning of epiboly. Formation of the embryo and migration of a layer of cells from the edge of the blastodisc to overgrow the yolk. Head and trunk region of embryo evident (a) (*left*) one-third epiboly. Embryonic axis near 6 o'clock. ×19 (formalin. (b) (*below*) one-third epiboly. Embryonic axis near 7 o'clock. Head and trunk region of embryo evident. ×22 (Stockard's).



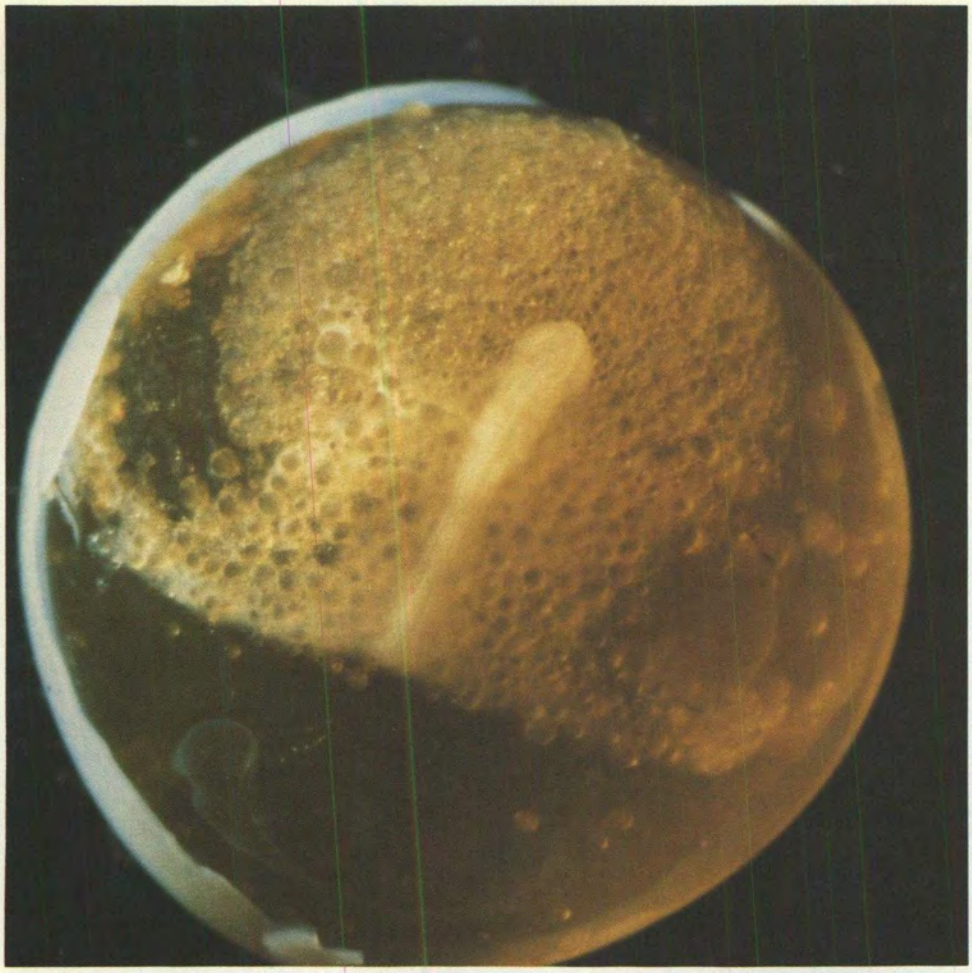


FIG. 13. Stage 15, rudiment of future tail near advancing border of germ ring (a) (*left*) two-thirds epiboly. Optic vesicles forming. ×19 (formalin), (b) (*below*) two-thirds epiboly, ×22 (Stockard's).

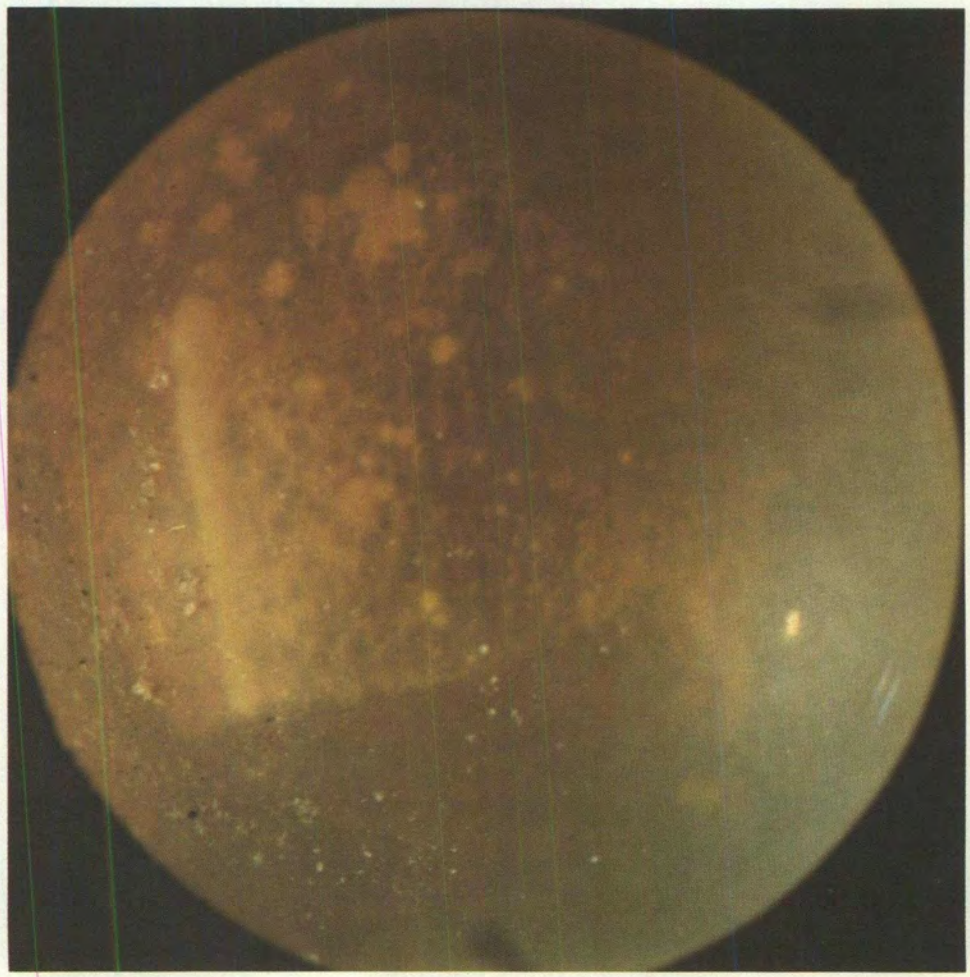


FIG. 14. Stage 16, epiboly near completion. Embryo established. Head near animal pole and presumptive tail at border of advancing germ ring (a) (*left*) blastopore near 9 o'clock. ×18 (formalin), (b) (*below*) blastopore near 6 o'clock. ×21.5 (Stockard's).

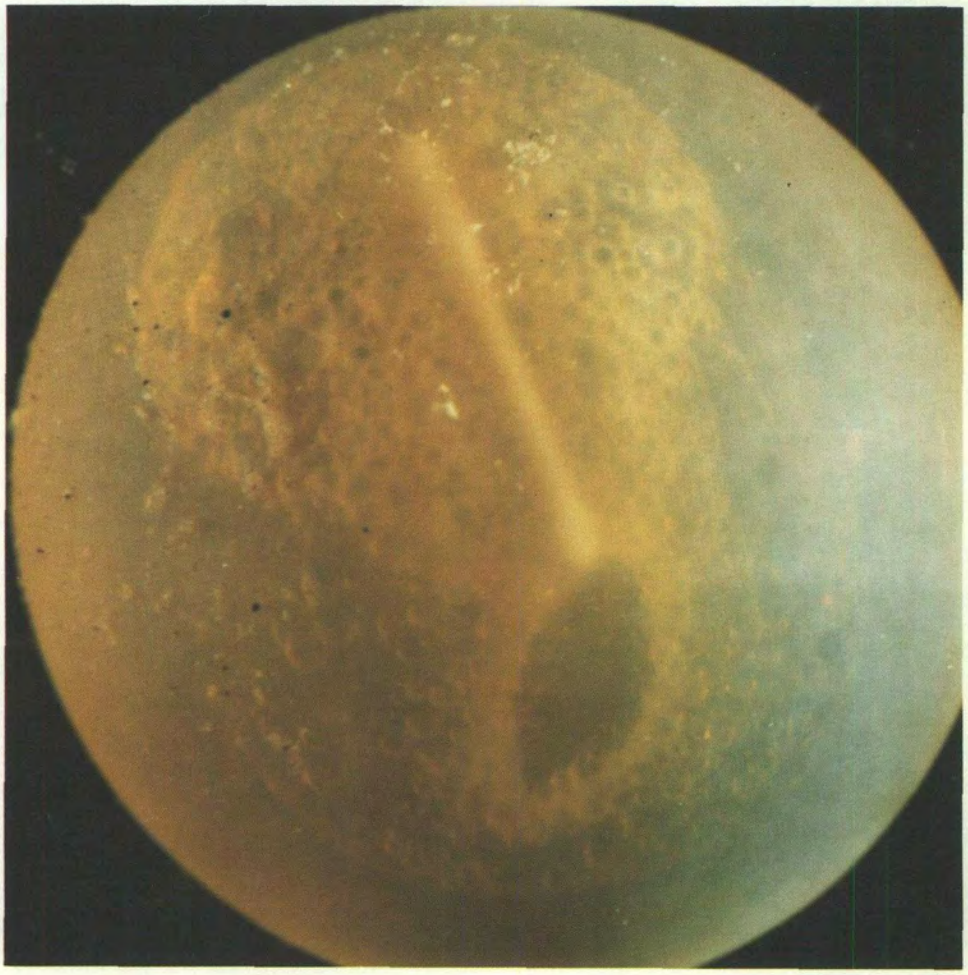


FIG. 15. (*left*) Stage 18, caudal bud formed and free from surface of yolk. Muscle somites in trunk region and lenses visible in eyes. ×22 (formalin).

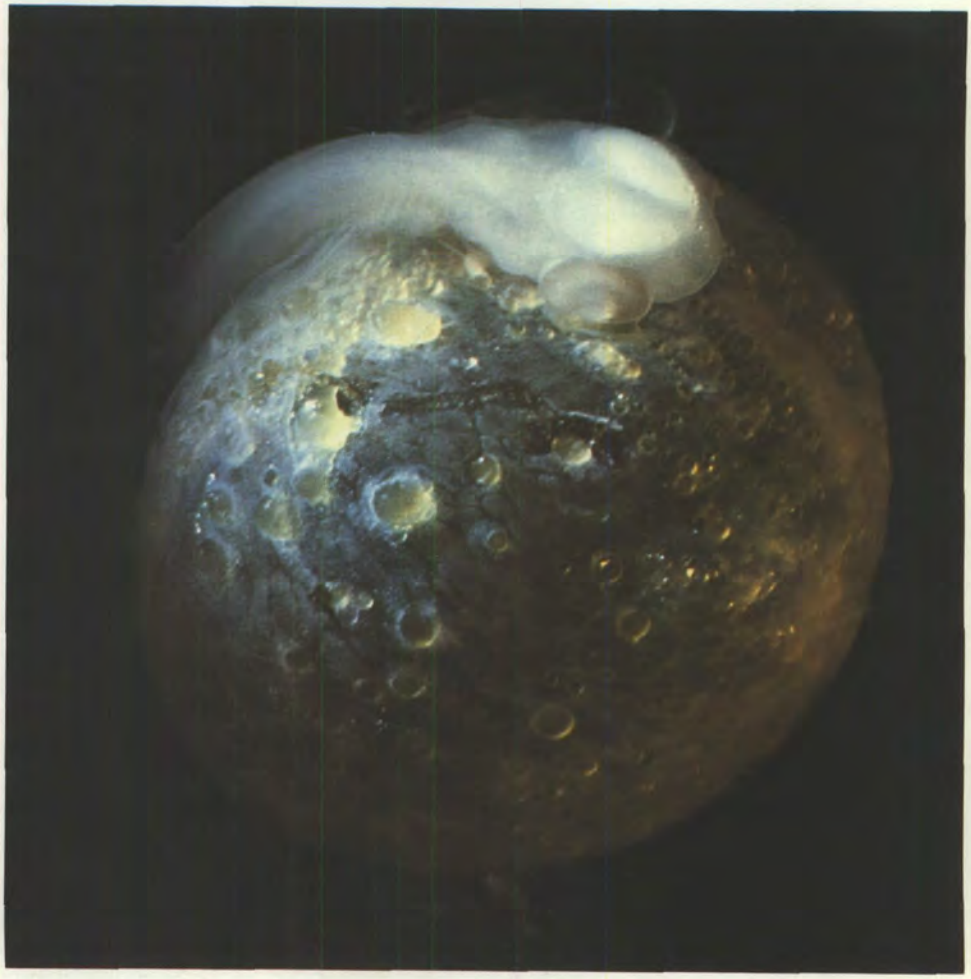


FIG. 16a (*right*) Stage 21, one-quarter vascularization of yolk, faint pigmentation of eyes most prominent around their perimeter. Pectoral fin buds present behind head of embryo. ×18 (formalin).

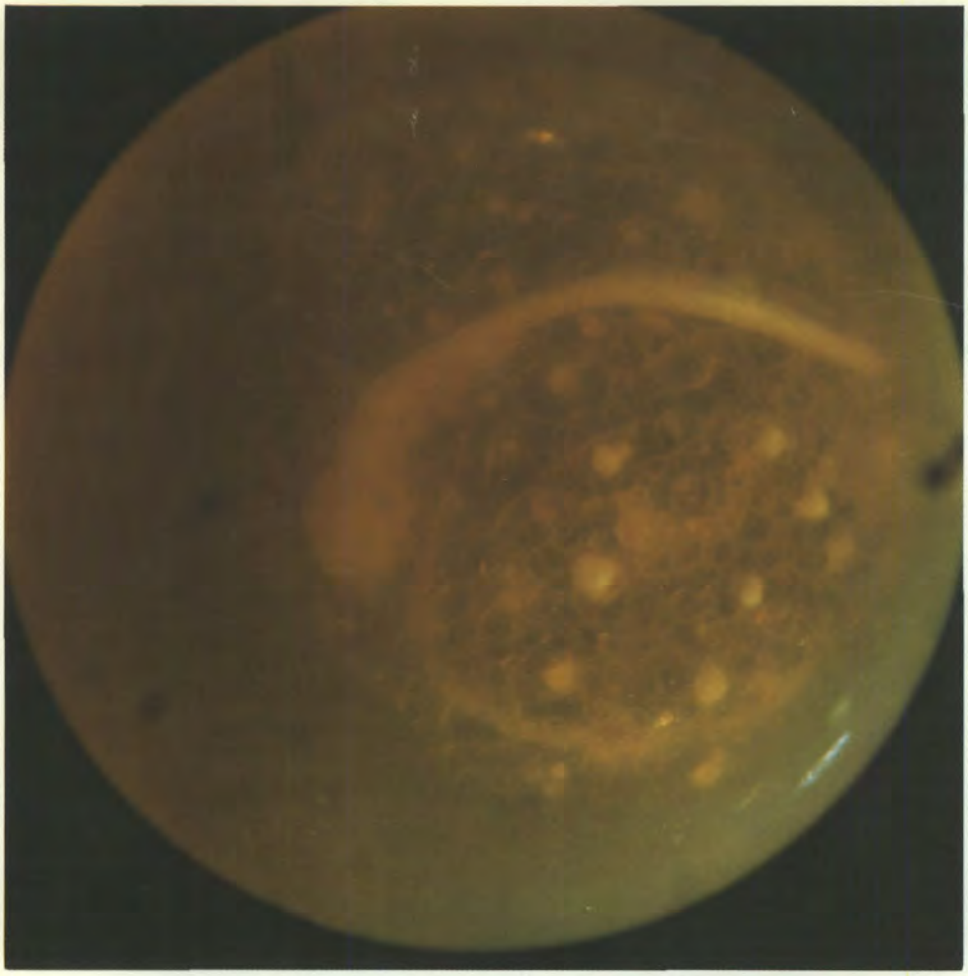


FIG. 16b. (*left*) Stage 21, one-quarter vascularization of yolk, vitelline vein at left side of embryo encircles about one quarter of yolk. ×21.5 (Stockard's).

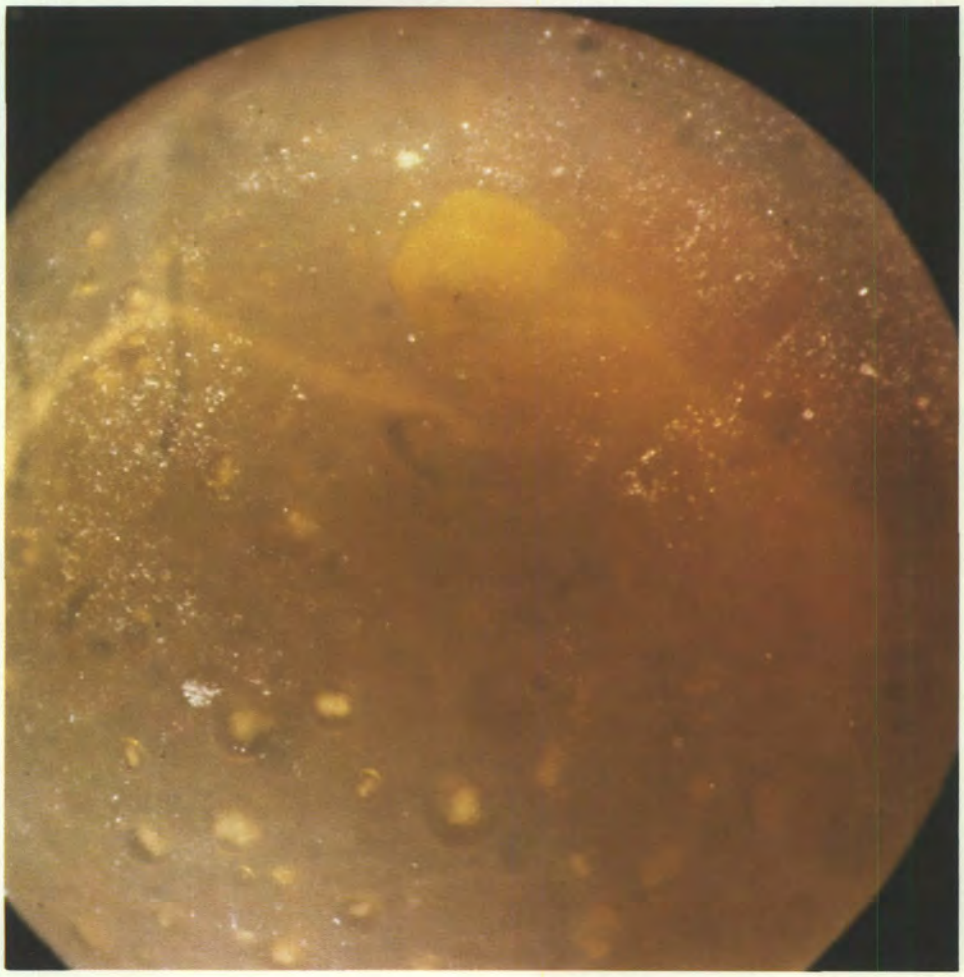


FIG. 17. (*right*) Stage 24, three-quarters vascularization of yolk. Vitelline circulation covers about three-quarters of yolk. Eyes fully pigmented and embryo is said to be "eyed." ×21.5 (Stockard's).

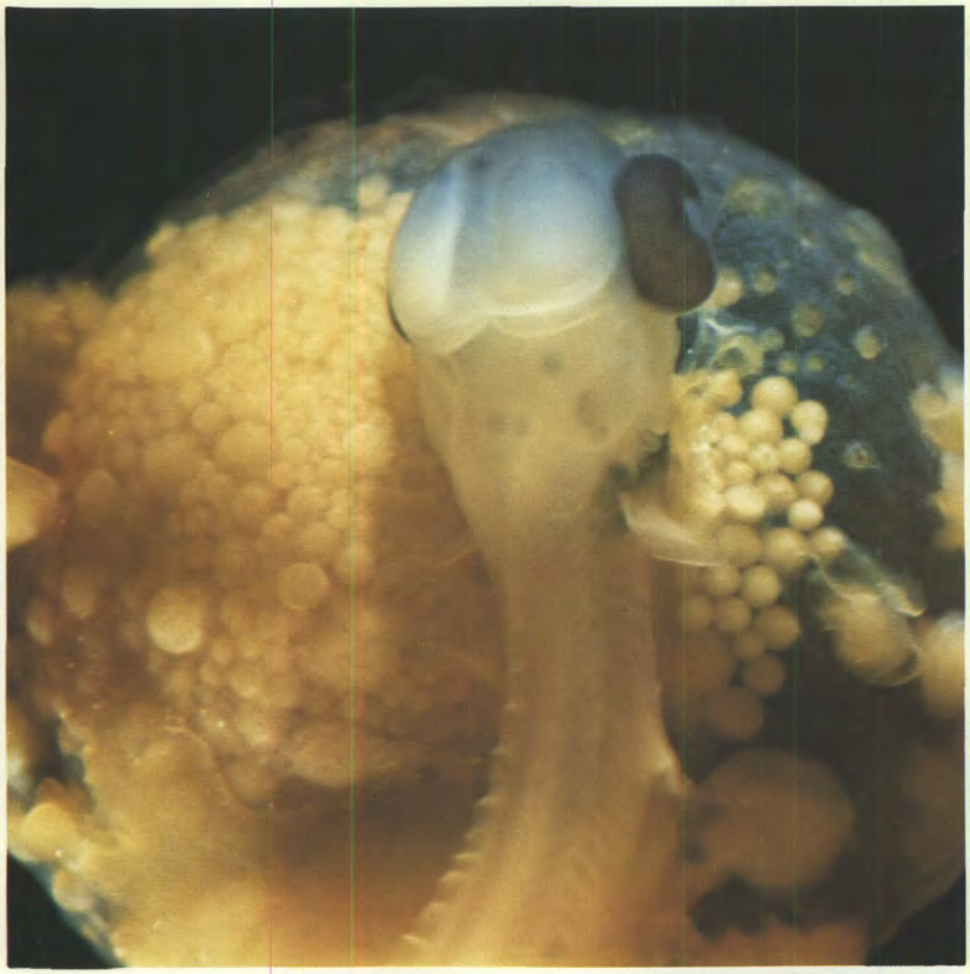


FIG. 18. (*left*) Stage 25, development of operculum or gill cover. Operculum not yet covering first branchial or gill arch. Also developing brain, muscle segments developing from somites, the paired pectoral fins and the fully pigmented eye. ×22.5 (formalin).

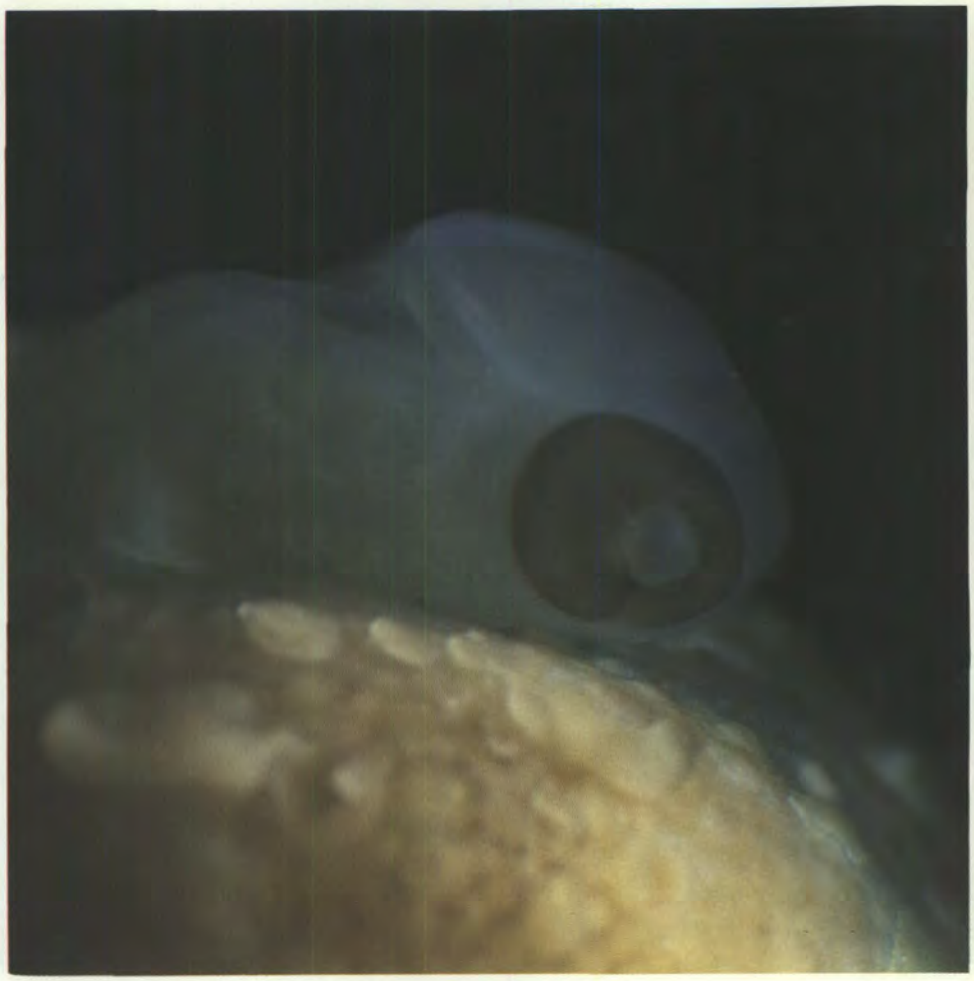
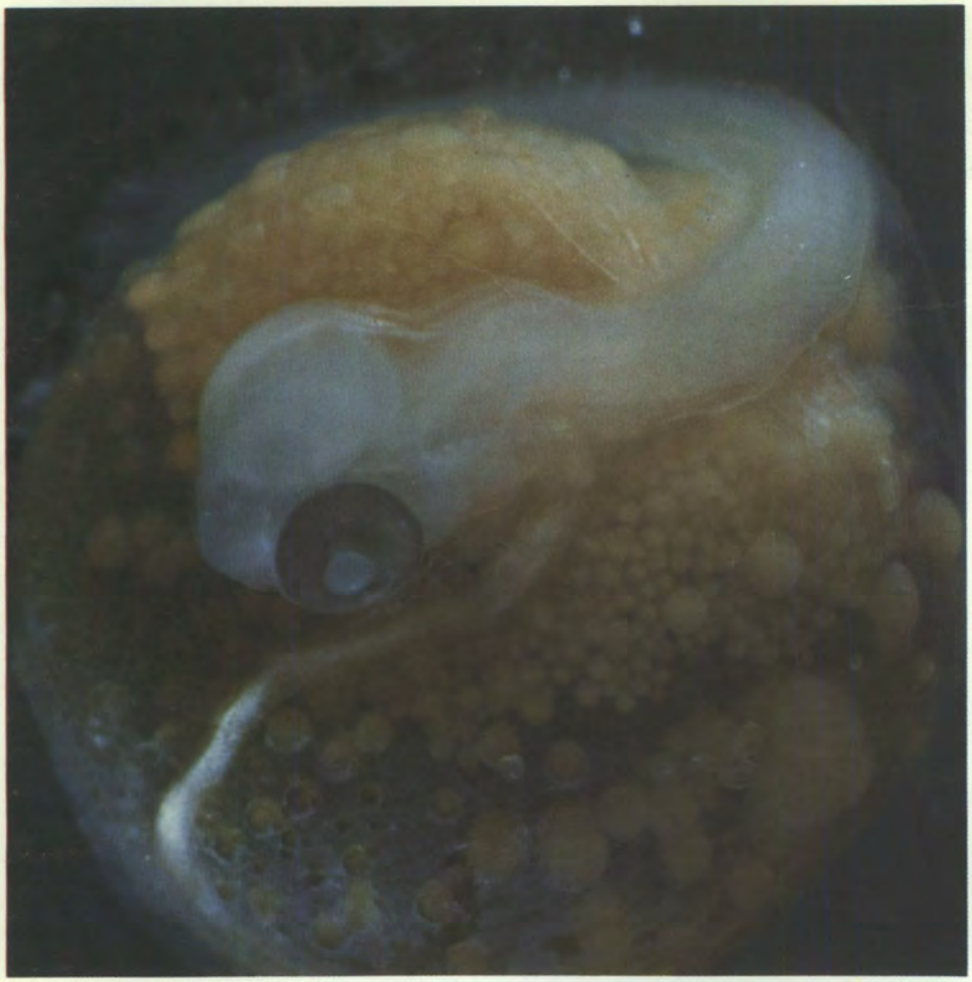


FIG. 19. (*right*) Stage 27, operculum covers first branchial arch. ×28 (formalin).



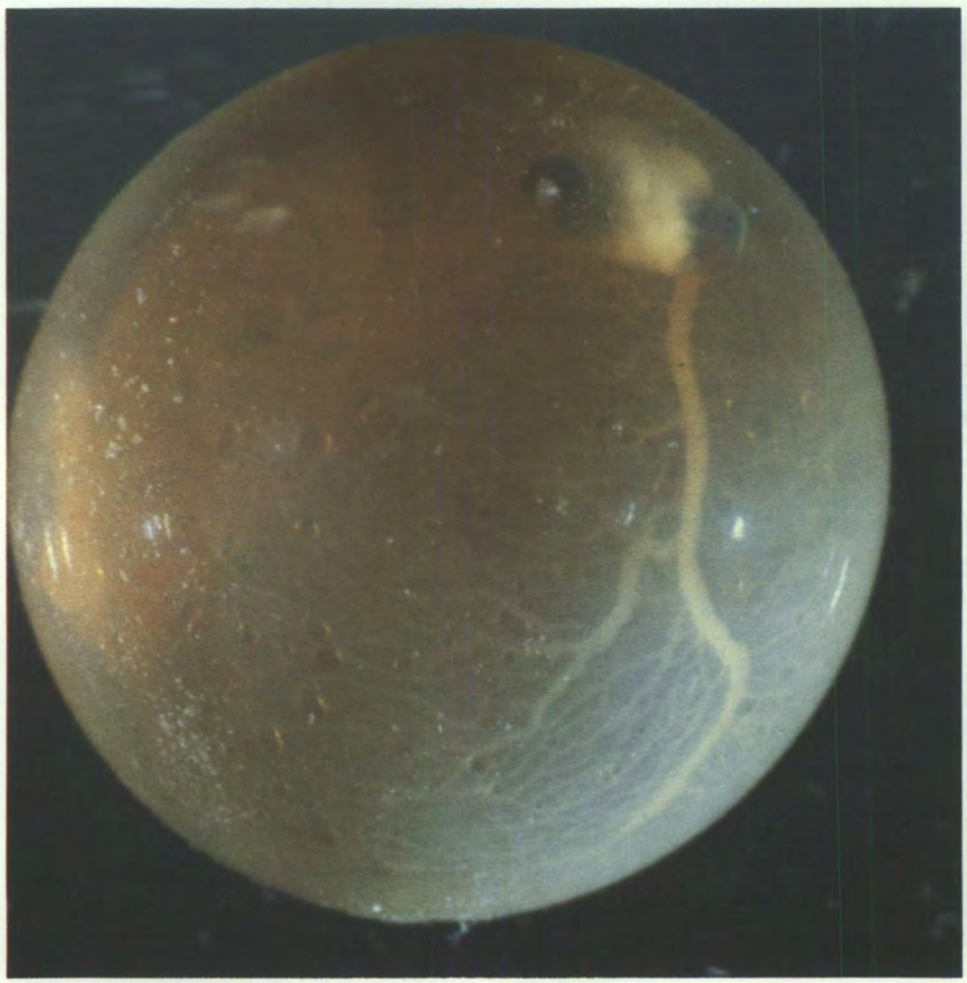


FIG. 20. Stage 30, ready to hatch (a) (*left*) operculum covers all gill arches. ×24 (formalin), (b) (*below*) yolk vascularization complete. ×19 (Stockard's).

TABLE 1. Embryonic development in the sockeye egg. At the left are 30 stages of development from fertilization to hatching, and characteristics identifying each stage (Vernier 1969). Three developmental phases are shown: cleavage (cell division), gastrulation (tissue formation), and organogenesis (organ formation). At the right are shown the times (hours, days, degree-days) to reach various stages at temperatures of 5, 8, and 11°C (test results) and intermediate temperatures of 6, 7, 9 and 10°C (interpolated from test results). All stages of development were not seen in the test series; the times for those not seen, shown in parentheses, are estimated from the remainder of the data.

Stages of development		Time to reach stage																			
		5°C			6°C			7°C			8°C			9°C			10°C			11°C		
Stage	Characteristics	Hours	Days	Degree-days	Hours	Days	Degree-days	Hours	Days	Degree-days	Hours	Days	Degree-days	Hours	Days	Degree-days	Hours	Days	Degree-days	Hours	Days	Degree-days
	Cleavage																				
1	Fertilized egg, no cell division																				
2	Two cells	15	0.6	3	13	0.6	3	12	0.5	3	10	0.4	3	9	0.4	3	8	0.3	3	7	0.3	3
3	Four cells	25	1.0	5	21	0.9	5	18	0.7	5	15	0.6	5	13	0.5	5	11	0.5	5	10	0.4	5
4	Eight cells	30	1.3	6	26	1.1	7	23	1.0	7	21	0.9	7	18	0.7	7	15	0.6	6	14	0.6	6
5	Sixteen cells	39	1.6	8	33	1.4	8	29	1.2	8	25	1.0	8	21	0.9	8	18	0.8	8	16	0.7	7
6	Thirty-two cells	46	1.9	10	40	1.7	10	35	1.5	10	31	1.3	10	26	1.1	10	22	0.9	9	19	0.8	9
7	Early morula, numerous visible cells	56	2.3	12	49	2.1	12	42	1.8	12	38	1.6	13	32	1.3	12	27	1.1	11	23	1.0	11
8	Late morula	64	2.7	13	56	2.3	14	49	2.0	14	43	1.8	14	38	1.6	14	33	1.4	14	30	1.2	14
9	Start of blastodisc expansion	120	5.0	25	100	4.2	25	84	3.5	24	71	2.9	24	61	2.5	23	52	2.2	22	45	1.9	21
10	Blastula	185	7.5	38	153	6.4	38	128	5.3	37	108	4.5	37	(94)	3.9	35	(80)	3.3	33	(68)	3.7	41
	Gastrulation																					
11	Terminal caudal bud	(260)	9	46	200	8.3	50	179	7.5	52	160	6.7	53	(132)	5.5	49	(110)	4.6	46	(113)	5.8	64
12	Rough outline of embryo	(310)	10	52	250	10	62	205	8.5	60	190	7.9	63	(164)	6.8	61	(133)	5.5	55	No data		
13	1/3 epiboly, embryo clearly visible	380	16	79	325	13	81	285	12	83	245	10	82	205	8.5	77	175	7.3	73	150	6.2	69
14	1/2 epiboly, first somites	550	23	115	450	19	112	380	16	111	310	13	103	280	12	105	235	9.8	98	212	8.8	97
15	2/3 epiboly	600	25	125	515	21	129	440	18	128	345	14	115	320	13	120	280	12	117	233	9.7	107
16	3/4 epiboly	630	26	131	550	23	137	470	20	137	375	16	125	340	14	127	290	12	121	250	10	115
17	Blastopore closed	670	28	140	580	24	145	505	21	147	390	16	130	375	16	141	328	14	137	280	12	128
	Organogenesis																					
18	Caudal bud free	780	32	160	660	27	162	550	23	160	425	18	142	395	16	148	340	14	142	298	12	137
19	Parts of brain district (metencephalon, myelencephalon)	805	33	165	670	28	167	(590)	25	172	455	19	152	(400)	17	150	(350)	15	146	325	13	149
20	Heart beats	825	34	170	680	28	170	(625)	26	182	485	20	162	(440)	18	165	(365)	15	152	338	14	155
21	Just eyed, 1/4 yolk vascularization	940	39	196	790	33	198	670	28	195	550	23	184	485	20	182	415	17	173	358	15	164
22	2/3 yolk vascularization	1150	48	240	950	40	240	800	33	233	665	28	224	570	24	214	475	20	198	428	18	196
23	Return of caudal blood supply to heart via only the cardinal veins																					
24	"Eyed," 3/4 yolk vascularization	1170	49	244	1030	43	258	895	37	261	780	32	256	740	31	277	645	27	269	530	22	243
25	Caudal flexing, anal fin started	1280	53	265	1100	46	275	965	40	281	820	34	272	(740)	31	277	(650)	27	271	575	24	264
26	Operculum covers part of first branchial arch, dorsal fin started	1500	62	310	1260	52	315	1060	44	309	910	38	304	(800)	33	300	(700)	29	292	740	31	339
27	Myotome buds in dorsal fin, operculum covers first branchial arch	1680	70	350	1330	55	332	1160	48	338	990	41	328	(880)	37	330	(770)	32	321	820	34	374
28	Pelvic fin buds, indentation in embryonic finfold marks start of caudal fin	1720	72	360	1490	62	372	(1250)	52	365	1105	46	368	(950)	40	356	(780)	32	325	870	36	396
29	Operculum covers second branchial arch, rays in caudal fin differentiating	(2040)	85	425	1620	67	405	(1650)	69	481	1210	50	400	(1200)	50	450	(1030)	43	429	910	38	418
30	Hatching, operculum covers all arches 50% hatch	2510 2856	105 119	523 595	2250 2550	94 106	562 636	(1810) 2200	75 92	528 642	1300 1920	54 80	433 640	(1340) 1700	56 71	502 637	(1170) 1500	49 62	487 625	1030 1368	43 57	472 627

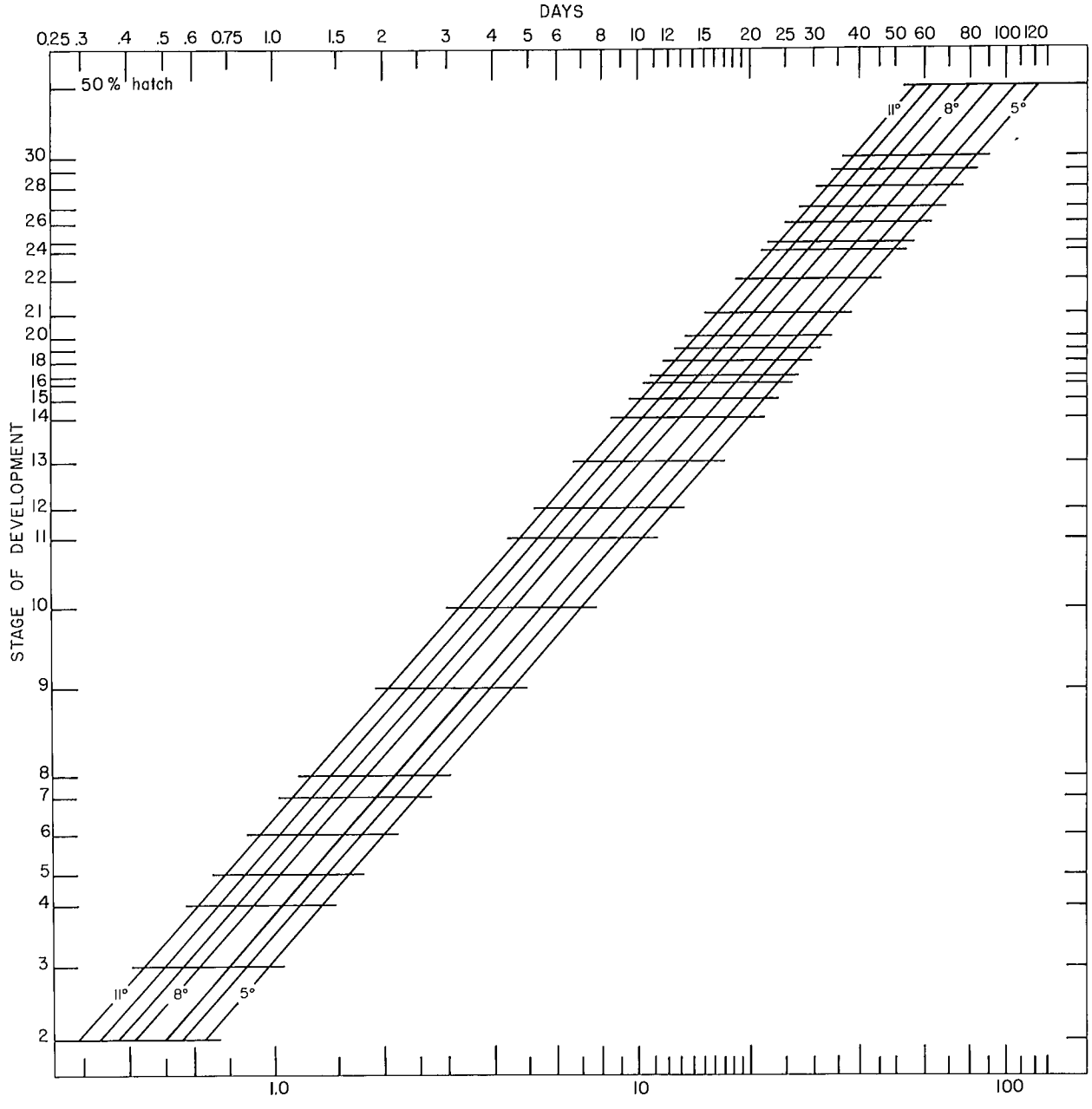


FIG. 21. Relation between incubation temperature and the time (days) to reach various stages of embryonic development. The oblique lines show the relationship at the three test temperatures (5, 8, 11°C) as well as the intermediate temperatures (6, 7, 9, 10°C) obtained by interpolation. If two of the three variables (stage of development, incubation time in days, temperature) are known, the third may be estimated.