ON THE UNINSEMINATED EGG AND THE EGG WITH DEAD EMBRYO OF DOG-SALMON, *ONCORHYNCHUS KETA* (WALBAUM)

By

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It has been accepted customarily that the eggs of salmonoid fishes always turn white or opaque when they die. However, the eggs which are not inseminated or the eggs with dead embryos do not slways turn white or opaque but retain normal translucent appearance for a long time. Why these eggs do not turn white or opaque is discussed in the present paper.

Before going further, the writer wishes to acknowledge his indebtedness to Prof. T. INUKAI of the Faculty of Agriculture, Hokkaido University, for his kind suggestions and guidance. The writer's thanks are also due to Mr. T. ARAI, chief of Hokkaido Salmon Hatchery and the members of the hatchery, Dr. S. SANO, Mr. K. SHIBATA and others who have supported the writer in various ways.

Uninseminated Eggs

In hatcheries and laboratories, uninseminated eggs are commonly found among fertilized ones, though they occur usually in very low percentage. The eggs, however, are very difficult to be distinguished by their appearance from the neighbouring inseminated eggs in the early period. They are distinguishable when the inseminated eggs enter in "eyeing" stage after about a month. When allowed to remain undisturbed, most of them maintain translucent appearance for a long time even after the hatching time of the inseminated eggs, though some of them turn white or opaque one by one.

In the present observation, eggs stripped from a mature female dog salmon, *Oncorhynchus keta* (WALBAUM) were divided into two groups, the inseminated and the uninseminated. They were reared separately in running water at 10 ± 1°C. Several eggs were picked out from the groups at certain intervals at the same time and fixed in BOUIN's solution. Then the blastodiscs

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were examined externally and histologically after removal of the chorions.

In the eggs before immersion into fresh water, the protoplasm of the blastodisc apreads widely and thinly over the animal pole, measuring about 3 mm in diameter (PLATE I, 1).

When the eggs are immersed into fresh water, the protoplasm of the blastodiscs of both groups begins to condense in the same manner. Slight condensation can be observed at about one hour after the immersion (PLATE I, 2 A, 2 B) and after five hours it progresses considerably (PLATE I, 3 A, 3 B). After about nine hours, the protoplasm forms a distinct blastodisc measuring about 1.5 mm in diameter, accomplishing bipolar differentiation. In the inseminated eggs, the first segmentation begins at this time (PLATE I, 4 A, 4 B). Until this time the blastodiscs of both groups go through apparently the same changes. The fact that the uninseminated egg is activated by immersion into fresh water has been reported in *Trutta iridea* by BEHRENS (1898), in *Salmo salvelinus* by RUNSTROEM (1920), in *Oncorhynchus keta* by YAMAMOTO, K. (1951), and by others.

In the inseminated egg, the first cleavage occurs between nine and eleven hours after insemination, the second twelve and fifteen hours, the third sixteen and nineteen hours, the fourth twenty and twenty-one hours and morula stage appears at about twenty-four hours. During this period the blastodisc rises up gradually from the yolk like a cap. The blastodisc of the uninseminated egg also rises up entirely in the same manner with that of the inseminated egg, though the cleavage naturally does not occur (PLATE I, 5 A～9 A, 5 B～9 B).

At about forty hours after insemination, the blastodisc of the inseminated egg shows the early blastula with typical round shape. The blastodisc of the uninseminated egg also affords the most condensed condition, measuring about 1 mm in diameter (PLATE I, 10 A, 10 B).

After about fifty-four hours, the blastula begins to flatten gradually extending its area until it becomes perfectly flat at about four days. In the uninseminated egg, the flattening of the blastodisc progresses in the same manner as the blastula (PLATE I, 11 A～14 A, 11 B～14 B).

At about seven days, the inseminated egg enters the gastrula stage, showing invagination at one part of the germ-ring and the blastoderm begins to extend over the yolk. At the same time, the blastodisc of the uninseminated egg begins to disperse and degenerate gradually, being invaded by oil-drops from the underlying yolk (PLATE I, 15 A, 15 B).

In the uninseminated egg the blastodisc at five days presents an apparently compact appearance and is homogeneous in structure (PLATE II, 1 A, 1 B). The blastodisc at seven days contains some small vacuoles. These are the oil-drops

originated from underlying oil-masses (PLATE II, 2 A, 2 B). At ten days (PLATE II, 3 A, 3 B) and also at eighteen days (PLATE II, 4 A, 4 B), the invasion of the oil-drops accompanying the degeneration of the blastodisc is progressed further. At twenty-six days the blastodisc has degenerated almost entirely and the protoplasm disperses widely over the animal pole (PLATE II, 5 A, 5 B).

As mentioned above, the blastodisc of the uninseminated egg performs the same change in contour as that of the inseminated egg during about seven days, while afterward it continues to degenerate.

The vitelline membrane of the egg, however, remains always intact and its property of impermeability to water and salts which was found by GRAY (1932) retains in spite of the degeneration of the blastodisc. This is the reason why the uninseminated egg maintains translucent condition for a long time without turning opaque.

However, the strength of the membrane seems to decrease with the lapse of time. The egg retained in the water for a long time turns opaque easily after slight mechanical irritation. When such eggs are examined after fixing BOUIN's solution, the vitelline membrane is found ruptured.

Eggs with Dead Embryos

Sometimes in hatcheries numbers of dead eggs appear at about one month after the insemination, turning opaque in succession. In such eggs embryos are not found at all as if they were uninseminated ones.

This phenomenon has been noticed by OKADA and MIURA (1939) who reported the eggs to be presumably inseminated ones of which embryos had been killed by certain causes e. g., suffocation in the early stages of the development. Recently, AFFLECK (1953) also has reported the same fact in the trout egg in Australia.

The following experiments were carried out to ascertain whether the phenomenon can be induced experimentally by means of suffocation.

Method of Experiment

Water with low oxygen tension was overflowed in a glass bottle of about 300 cc capacity and the eggs at 30 minutes after insemination were filled in to about half capacity of the bottle (about 7 egg layers and about 5 cm in thickness). The bottle was stoppered tightly with a cork so as not to allow air bubbles to remain in it and immersed in running water at 10 ± 1°C for a certain time. Then the eggs were transferred again into running water and reared as usual. Some of the eggs were fixed from time to time in BOUIN's solution to examine the change of the blastodisc. The dead eggs which had turned opaque were

counted once a day. The low oxygen water was produced by boiling water for several minutes and cooling it slowly in connection with the oxygen absorbing bottle which contains alkaline pyrogallol. At the same time, a half of the inseminated eggs were reared as control.

Experiment I.

Time treated in the suffocation bottle: 24 hours.
Oxygen tension of the water used: 0.94 cc/1.
Degree of saturation: 11.9 % (10°C).

Result of experiment: The number of dead eggs which turned opaque previous to the "eyeing" stage is shown in Table 1.

TABLE 1.

Days after insemination	Treated eggs	Control
0	Suffocated for 24 hrs	0
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	1	0
14	0	0
15	5	0
16	15	0
17	32	0
18	37	0
19	36	0
20	29	0
21	7	0
22	5	0
23	6	0
24	9	0
25	5	0
Total number	266	about 300
Dead eggs	187	0
Percentage	70.3	0
Remaining eggs	79	about 300
Percentage	29.7	100

When released from the suffocated condition, the eggs remained for some time without showing any change in appearance. Then, after about two weeks they began to turn opaque in succession and finally about 70 % of them turned opaque before the "eyeing" stage. No embryo was observed in these eggs at all.

The number of eggs which had normal appearance after 25 days were 79 in total. They were all fixed in BOUIN's solution and examined.

Eggs with normally developed "eyeing" stage . . .	23 (29 %)
Eggs of slightly delayed development	2 (3 %)
Eggs with stunted and deformed-embryo	6 (7 %)
Eggs without embryo	48 (61 %)

When the non-embryo eggs mentioned above are included in the category of dead eggs, the percentage of dead eggs which seem to be uninseminated rises to about 90 %.

In the control, the eggs at twenty-four hours after insemination show thirty-two cells stage. In the suffocated group, the eggs in the upper part of the suffocation bottle are somewhat delayed in development, while the eggs at the bottom show distinctly poor development.

At ten days after release in the running water, a few in the group of the eggs which were situated at the upper layers develop normally while the rest are all stunted or degenerating. Probably the former was supplied with some oxygen from the upper water layer left in the suffocation bottle, while the latter was almost perfectly in suffocated condition without supply of oxygen.

The influence of suffocation upon the developing eggs of fishes has been studied by LOEB, J. (1894, '95) in detail on those of *Ctenolabrus* and *Fundulus*. OKUBO (1949) has reported briefly on the influence in the early development of the dog-salmon. The results of these authors agree with that of the present writer in the point that the segmentation is disturbed and hindered remarkably.

After the release in the running water, most of them can not recover from the effect of the suffocation and the embryos begin to degenerate by and by as seen in the uninseminated egg. It can not be demonstrated now, however, why some of them turn opaque after comparatively short time contrary to the case of the uninseminated eggs though their vitelline membrane is ruptured.

Experiment II.

In the preceding experiment, the effect of the suffocation treatment might be insufficient as some of the eggs had lived for some time in the running water. Therefore, the present experiment was carried out under more intensive suffocation.

Time treated in the suffocation bottle: 72 hours.
Oxygen tension of the water used: 0.31 cc/1.
Degree of saturation: 3.9 % (10°C).

Result of experiment: The number of dead eggs which turned opaque previous to the time corresponding to the "eyeing" stage in the control are shown in Table 2.

TABLE 2.

Days after insemination	Treated eggs	Control
0	Suffocated for 72 hrs	0
1		0
2		0
3	0	0
4	0	0
5	0	0
6	0	0
7	2	0
8	0	0
9	1	0
10	0	0
11	0	0
12	0	0
13	1	2
14	0	1
15	0	0
16	1	0
17	0	0
18	1	1
19	2	0
20	1	0
21	1	0
22	3	0
23	0	0
24	2	0
25	0	0
Total number	340	about 300
Dead eggs	15	4
Percentage	4.4	
Remaining eggs	325	about 300
Percentage	95.6	

In the present experiment, dead eggs which turned opaque were only less than 5% while the rest retained normal translucent appearance. One hundred eggs among the latter were fixed in BOUIN's solution and examined. They all showed the blastodisc like that of the uninseminated eggs which passed the

same number of days in the water; their blastodiscs were degenerated entirely by the invasion of oil-drops. Naturally as these eggs did not hatch out, the mortality was 100 % after all.

Most of the remmaining eggs retain normal translucent appearance even after the hatch out of the control, though they easily turn opaque when subjected to slight irritation.

The four days old egg in the control enters late blastula (PLATE III, 1 A) while the egg at the same age in the experiment shows the blastodisc like that of uninseminated egg remaining for the same period in water. The surface of the former looks not so smooth as the latter (PLATE III, 2 A).

As observed by section, the blastodisc of the control contains many cells in it, being enveloped with a simple epithelium of periderm (PLATE III, 1 B) while that of the uninseminated egg shows only homogeneous structure of protoplasm (PLATE II, 1 B). The blastodisc of the experimented egg shows the degenerating process of cells as the result of treatment (PLATE III, 2 B).

In eight days old egg in the experimented group, the blastodisc is invaded by some of the oil gobules (PLATE III, 3 A, 3 B). This agrees with the fact that the degeneration of the blastodisc begins at about seven days in the uninseminated eggs (PLATE II, 2 A, 2 B).

The blastodiscs of sixteen days old egg (PLATE III, 4 A, 4 B) and twenty-four days old one (PLATE III, 5 A, 5 B) degenerate more and more in the same manner as that of uninseminated ones.

In such eggs, however, vitelline membrane remains entirely intact. Therefore, the egg remains translucent for a long time without turning opaque. When the egg turned opaque for some certain cause, vitelline membrane is found always ruptured.

Conclusion and Summary

The blastodisc of an uninseminated egg undergoes a similar change in contour to that of an inseminated one in early stages, though it naturally does not segment. At about seven day after immersion in fresh water, however, it begins to degenerate gradually as a result of invasion of underlying oil-globules. After about two weeks, the protoplasm of the blastodisc is dispersed widely over the animal pole and its distinct contour is lost almost entirely. In such egg, vitelline membrane remains quite sound and retains the property of impermeability to water and salts. This is the reason why the uninseminated egg shows translucent appearance for a long time without turning opaque. That is to say, vitelline membrane sometimes retains normal condition morphologically

and physiologically in spite of the degeneration of the blastodisc. When vitelline membrane is ruptured, the egg turns opaque.

When the inseminated egg in early stage is perfectly killed by suffocation, the blastodisc degenerates following the same course as that of the uninseminated one. In this case too, vitelline membrane remains intact and the egg does not turn opaque for a long time.

From the facts mentioned above, it is clear that the egg does not always turn opaque even if the blastodisc has degenerated. This suggests that physiologically vitelline membrane may be independent of the blastodisc.

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PLATES

PLATE I.

Morphological changes of the blastodiscs of the inseminated egg (A-series) and the uninseminated egg (B-series). (× 11, only 15 A × 9)

Fig. 1. Blastodisc of the freshly taken out egg before immersion into fresh water.

Fig. 2. Blastodisc at one hour after immersion into water.

Fig. 3. At five hours.

Fig. 4. At nine hours.

Fig. 5. At eleven hours.

Fig. 6. At fifteen hours.

Fig. 7. At nineteen hours.

Fig. 8. At twenty-one hours.

Fig. 9. At twenty-four hours.

Fig. 10. At forty-two hours.

Fig. 11. At fifty-four hours.

Fig. 12. At sixty-six hours.

Fig. 13. At seventy-eight hours.

Fig. 14. At four days.

Fig. 15. At seven days.

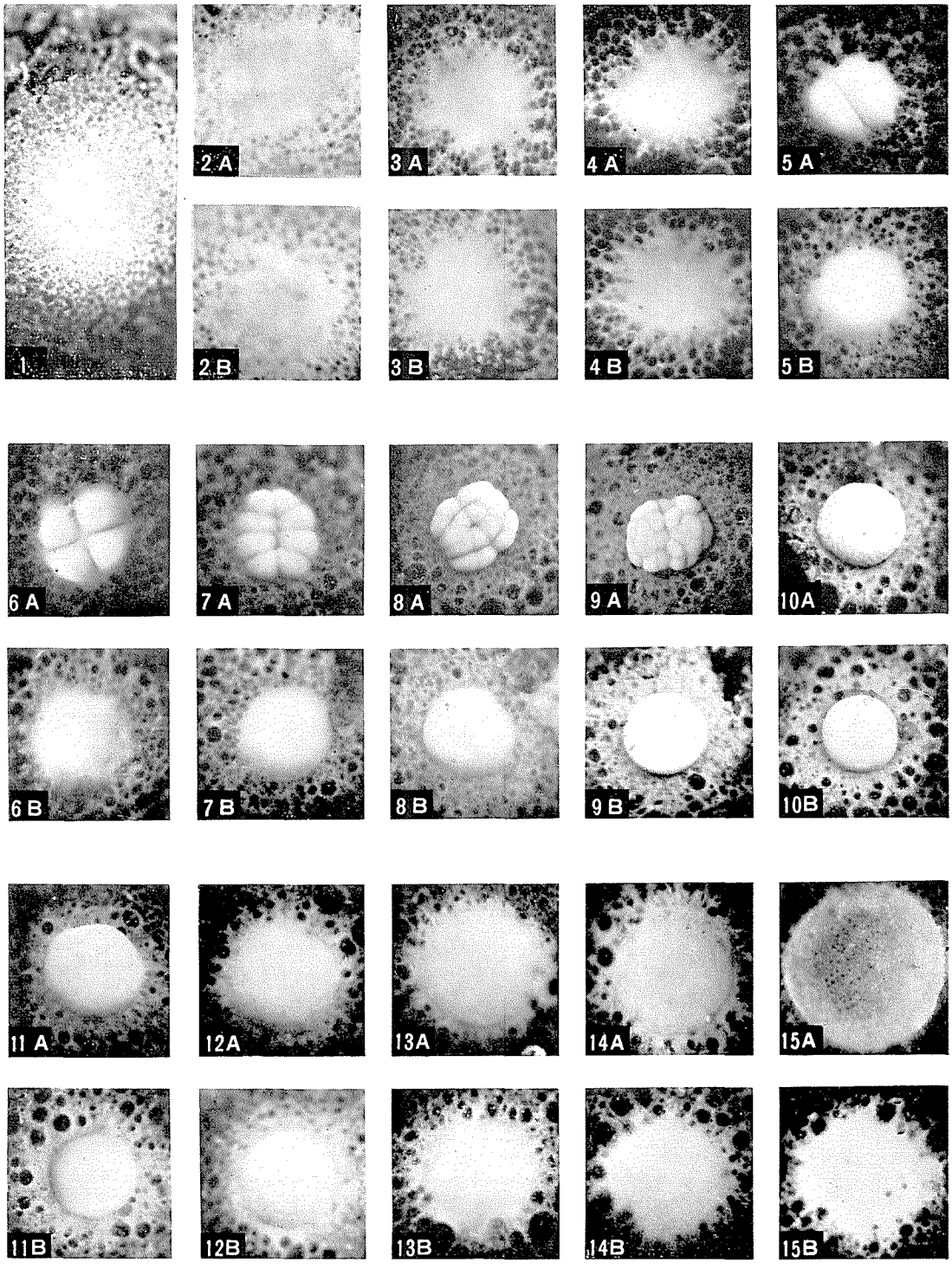

1
2 A
2 B
3 A
3 B
4 A
4 B
5 A
5 B
6 A
6 B
7 A
7 B
8 A
8 B
9 A
9 B
10A
10B
11 A
11B
12A
12B
13A
13B
14A
14B
15A
15B

PLATE II.

Degeneration of the blastodisc of the uninseminated egg, surface view (A-series, × 12) and sectioned B-series, × 43).

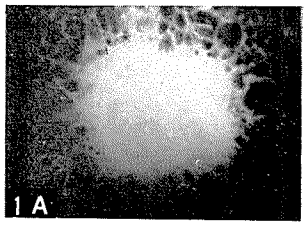
Fig. 1. Blastodisc at five days after immersion into fresh water.

Fig. 2. Blastodisc at seven days.

Fig. 3. Blastodisc at ten days.

Fig. 4. Blastodisc at eighteen days.

Fig. 5. Blastodisc at twenty-six days.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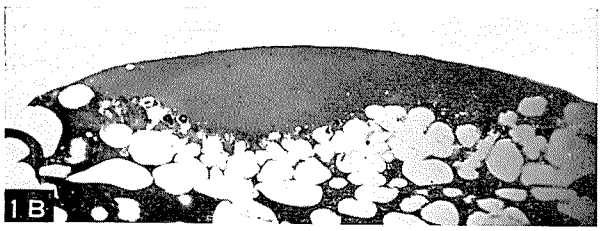
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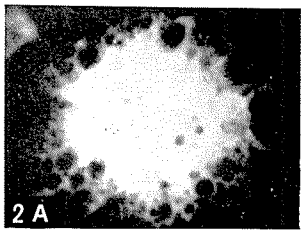
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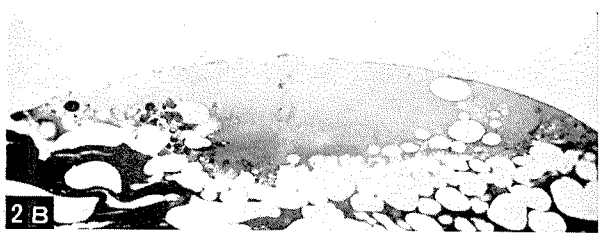
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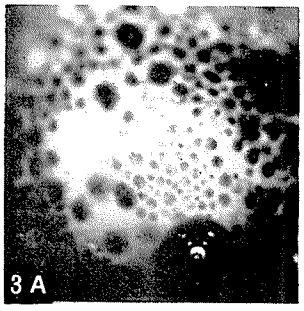
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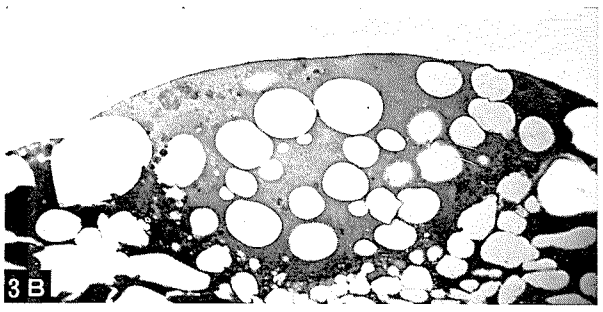
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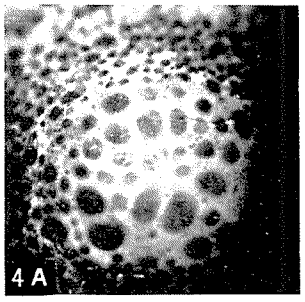
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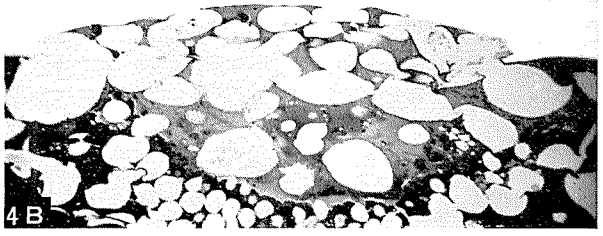
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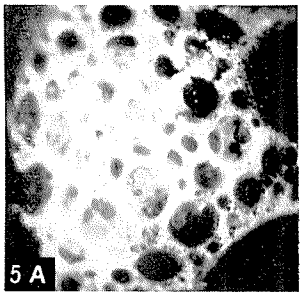

5 A


5 B

PLATE III.

Degeneration of the blastodisc of the suffocated egg, surface view (A-series, × 12) and the sectioned (B-series, × 43).

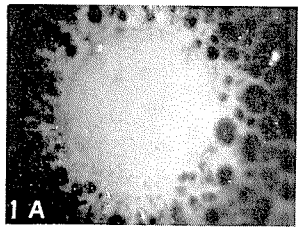
Fig. 1. Normal blastodisc of the inseminated egg at four days after the insemination.

Fig. 2. Blastodisc of the suffocated egg at the same number of days after the insemination.

Fig. 3. Blastodisc of the suffocated egg at eight days.

Fig. 4. Blastodisc of the suffocated egg at sixteen days.

Fig. 5. Blastodisc of the suffocated egg at twenty-four days.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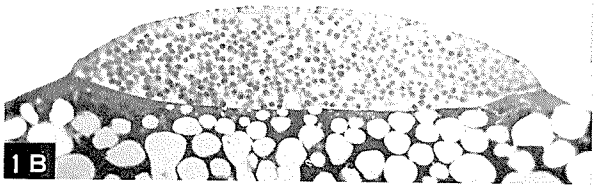
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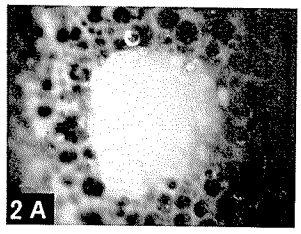
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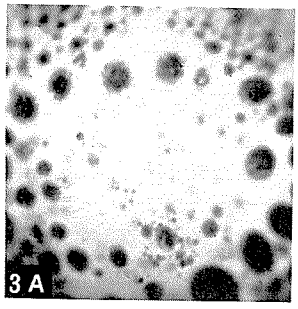
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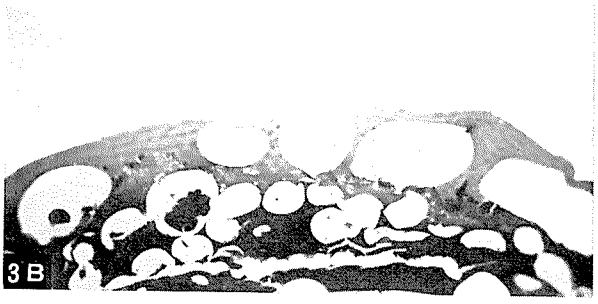
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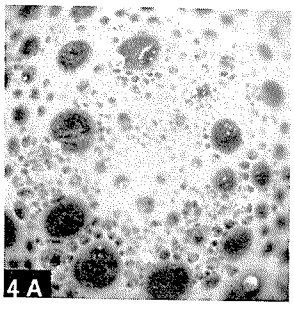
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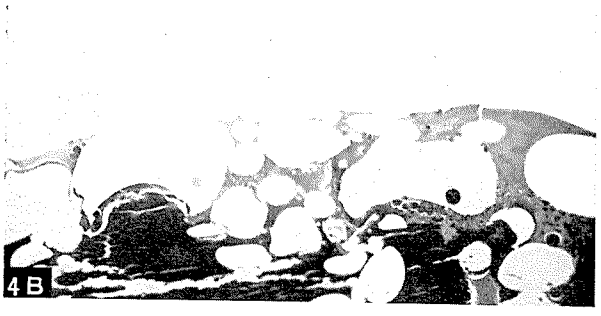
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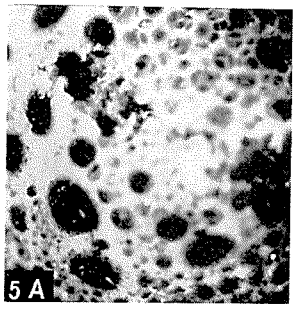
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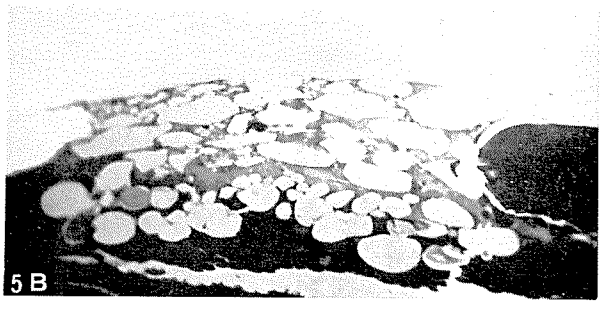
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