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Studies on Rainbow Trout Egg (*Salmo gairdnerii irideus*)

IV. Changes of Yolk Content During Embryogenesis

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(Figs. 1–5; Table 1)

In the first report of our work it was established that the contents of the yolk could be separated by centrifugation into three fractions[1]. The top layer was the oil globule colored by carotenoid pigment, the hypophasic solution (HDF) consisted of protein, and a small amount of material (LDF) between the two. Several points remain, however, which are not yet understood for their properties and physiological significance.

At least three proteins have been isolated from the yolk of the unfertilized egg of salmonoid[2~7]. They are lipoprotein, phosphoprotein, which has not yet been identified so far. In any case, protein is a predominant constituent of the salmonoid egg. At the early stage of development the amount of non-proteic nitrogen in the yolk sac is very small, but it increases with the growth[8].

The salmonoid egg has been physiologically examined by many investigators. The changes of the general composition[8~12], amino acids[3,13~16], lipids[10,12,17~25], and phosphorus compounds[26,27] in various developmental stages have also been studied, however, very little is known about the biochemical embryology of fish egg. Therefore, in this paper the changes of the yolk contents have been reported in order to obtain more information on the chemical transformations which occur during the utilization of the yolk reserve.

MATERIALS AND METHODS

The unfertilized and fertilized eggs of rainbow trout were obtained from the Zaoh Rainbow Trout Breeding Station, Miyagi-prefecture in January of 1966. The latter ones were brought to the Fishing School in Sendai, and kept in a breeding tank at 12–13.5°C of water temperature throughout the development of 45 days. The embryonic eggs were examined 3 hours after fertilization, then subsequently

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Abbreviations: HDF, high density fraction
LDF, low density fraction

8th, 13th, 23th, 28th (immediately after hatching), 32th, 40th, and 45th day.

The yolk sac of the eggs or alevins were punctured with a needle and their contents were completely rinsed out with 2 % saline solution. The effluents were centrifuged at chill temperature and separated into the three fractions: the oil globule, the hypophasic layer (HDF), and the small amount of LDF present in the intermediate zone. The oil globule was dissolved in petroleum ether, filtered off, then filtrates were concentrated under reduced pressure. LDF was weighed in order to be included under the head of embryo and chorion.

The hypophasic yellowish layer (HDF) was separated into protein and non-protein fractions by means of addition of 10 % trichloroacetic acid (TCA). The bound-lipid with protein in HDF was extracted by the BLIGH *et al.* process[28]. Nitrogen was determined by the micro-Kjeldahl technique.

The Tiselius electrophoresis was carried out as follows. The yolk content was homogenized with a carbonate buffer at pH 9.8, HDF was separated from the oil globule and LDF by centrifugiug. Thereafter it was dialyzed against the same buffer at 5°C for two days and then applied to the Tiselius electrophoretic apparatus of Hitachi HT-D type with a current of 5 mA/cm², for 30 minutes. The protein components were calculated from the proportions of each area by migrating boundaries.

RESULTS

The changes in weight, the moisture content of the egg and the alevin at the developmental stages are indicated in Table 1. The water content of the egg rapidly increased immediately after the fertilization but remained still before hatching, thereafter it abruptly decreased to be followed by a gradual increasing again. The change of egg weight showed a similar tendency to that of moisture. However, during all stages of development the dry weight continuously decreased.

Table 1 Weight changes of rainbow trout egg or alevin during development.

No.	Date of sampling	Stage	Time after fertilization	Wet weight * (mg)	Dry weight * (mg)	Moisture (%)
1	Jan. 23	Unfertilized	—	143.3	56.0	60.9
2	23	Fertilized	3 hrs.	151.1	52.9	65.0
3	31		8 days	152.2	52.9	65.2
4	Feb. 5	Eyed stage	13 days	151.1	52.6	65.2
5	10		18 days	149.4	52.6	64.8
6	15	Before hatching	23 days	149.7	52.2	65.1
7	20	After hatching	28 days	128.5	49.4	61.6
8	25		33 days	138.9	50.0	64.0
9	Mar. 4		40 days	164.8	47.0	71.5
10	9	Fry	45 days	178.2	45.6	74.4

* an average of every 50 eggs or alevins.

This may be considered as largely responsible for the steady reduction of HDF and the oil globule during the development as seen in Figures 1–A and 2–A.

The HDF weight of the yolk remained unchanged until the hatching stage then gradually decreased. The amount of HDF–nitrogen seemed to vary concomitantly with the weight of HDF. However was a temporary increment in nitrogen content of HDF (Figure 1–C).

The quantity of the oil globule (5.7 mg/egg) gradually decreased to 3.9 mg after hatching. However, bound-lipid, which is the lipid of lipoprotein in HDF,

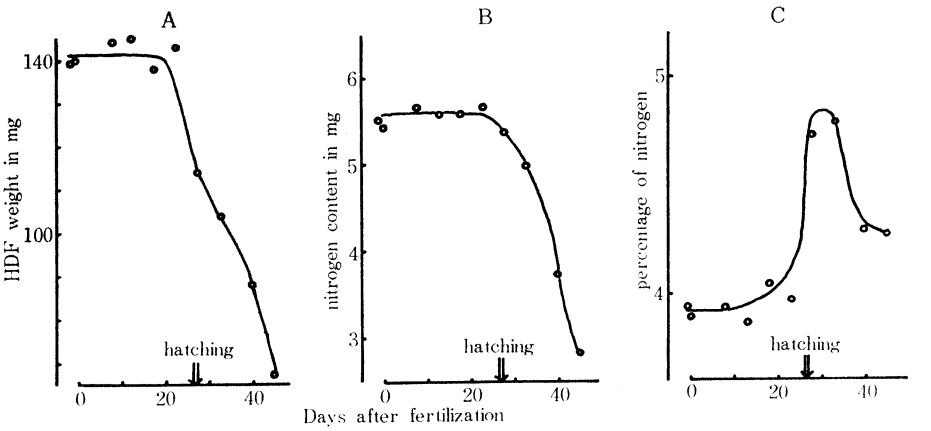

Fig. 1 Changes of HDF and nitrogen during development.

A HDF weight in one yolk
B HDF-nitrogen in one yolk
C Ratio of nitrogen in HDF

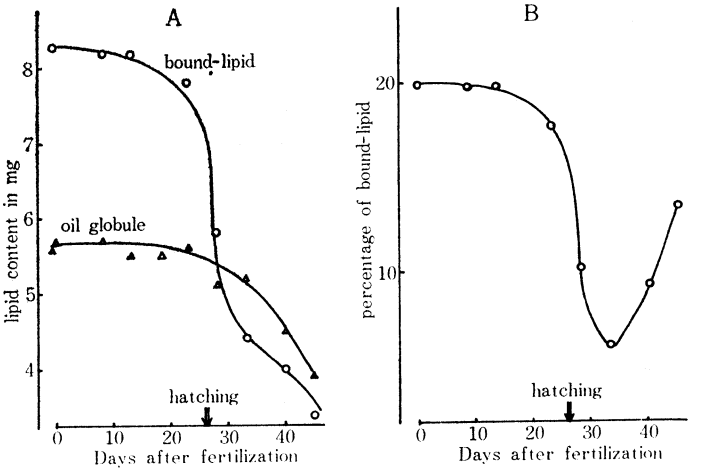

Fig. 2 Changes of lipids during development.

A Oil globule and bound-lipid per one egg or alevin
B Ratio of bound-lipid in TCA-insoluble matter

decreased rapidly to the same level as the oil globule. By the end of the examination time the former became lower than the latter (Figure 2–A). It was found that the change of the lipid content of TCA–insoluble matter of HDF occured in some degree throughout the embryogenesis, namely it was almost constant until 20th day, after which it rapidly came down to a level of 12 %, to go up subsequently again to 17 % (Figure 2–B). The decrease of lipid content of

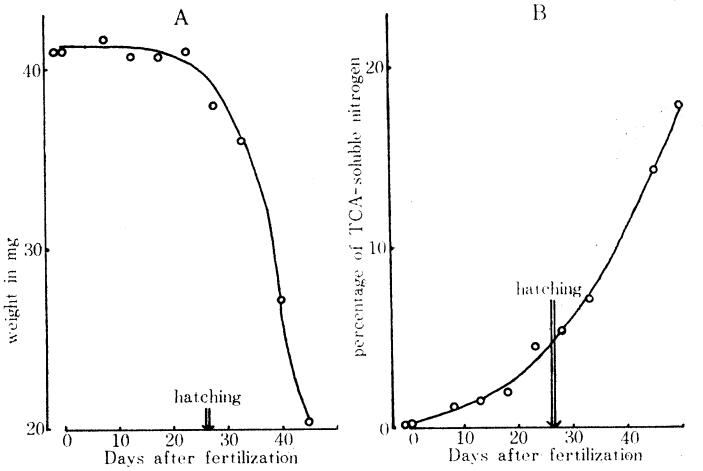

Fig. 3 Changes of TCA-insoluble and soluble matter in HDF during development
A Weight of TCA-insoluble matter per one yolk
B Ratio of TCA-soluble nitrogen in HDF-nitrogen

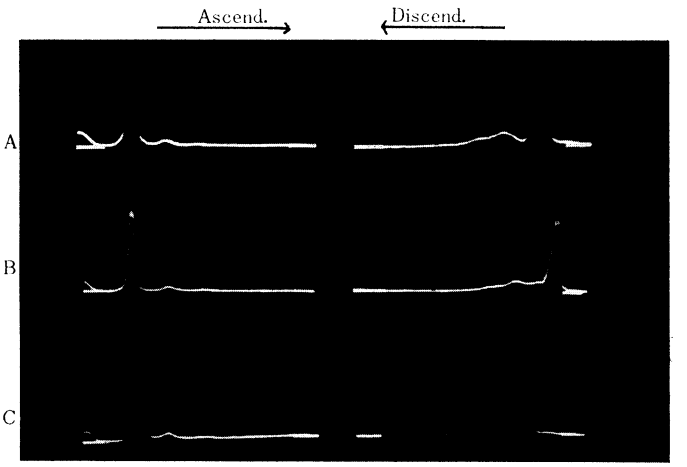

Fig. 4 Tiselius electrophoretic diagrams of yolk protein at different developmental stages, pH 9.8 in carbonate buffer (i=0.1), 5 mA/cm², for 30 mins.
A Before fertilization
B 23 days after fertilization (before hatching)
C 45 days after fertilization (after hatching)

lipoprotein at the hatching stage seemed to be closely related to the temporary increment of HDF-nitrogen (Figule 1-C). The consumption ratio of the bound-lipid was higher than that of the oil globule.

The changes of protein and non-proteic substances in HDF of one yolk are shown in Figure 3. The depression of TCA-insoluble matter of HDF in the yolk initiated at the time immediately after hatching. Thus, it was deduced that the protein was converted to non-proteic substances from an early stage of development, and this conversion continued till the later stage, as shown in Figure 3-B.

The electrophoretic diagrams displayed usually three peaks of protein (Figure 4). No difference was found in their proportion during the development. They were distributed in a ratio of $83.2 \pm 1.9\,\%$, $11.0 \pm 2.6\,\%$, and $5.8 \pm 1.8\,\%$, respectively, as shown in Figure 5.

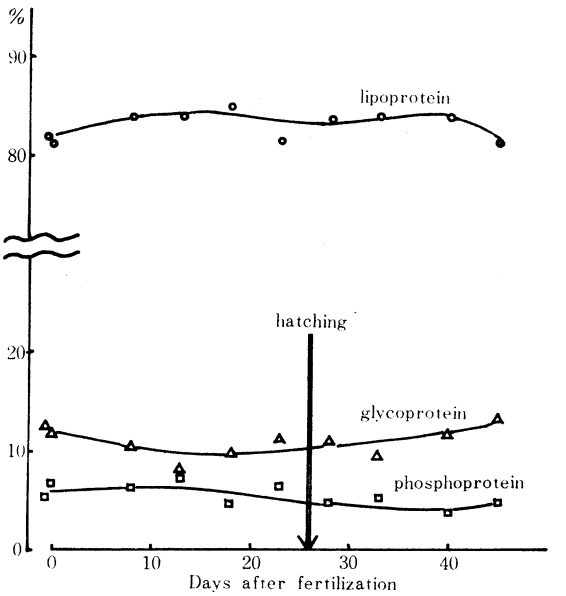

Fig. 5 Changes of protein components during development.

DISCUSSION

The biology of salmonoid eggs has been investigated minutely for a long period[29~33]. It is well known that the swelling of the egg by uptake of water is dependent on the process of embryogenesis, though the perivitellin fluid is lost by the rupture of the egg capsule at the hatching stage. The dry weight of the egg remained almost constant until the hatching period. A remarkable decrease was found at the stage of alevin, this phenomenon was supposed to be caused by the consumption of the yolk content.

The HDF-nitrogen contributed mainly to the protein, which took a greater

part of it in the yolk at the early stage. Protein is also a dominant constituent in food stuff for the embryo. It was suggested that an increase of non-proteic nitrogen in the yolk sac, especially after hatching, meant an appearance of free amino acids coming from the protein. May be for this reason the protein was kept as an important reserve in the yolk sac before utilization. In spite of the marked decrease of protein content in the yolk, it was observed by the electrophoretic analysis that three proteins were almost constant in their ratio throughout the development. Therefore, it seemed conceivable that all the proteins were equally converted to low molecular products.

The egg lipid consisted of two different fractions; one was the oil globule existing in free form, the another the bound-lipid which was easily split off from the lipoprotein by organic solvent. The oil globule was mainly used after hatching, but its consumption rate was less than that of the bound-lipid during development. The bound-lipid was preferentially consumed by the embryo rather than the oil globule. Therefore, both lipids of the egg should not be considered identical in their physiological signification.

The main change in yolk content occurring after hatching depended on the absorption of the proteins by the growing sac fry. It was reported that there occurred a steady increase in non-proteic nitrogen throughout the development[8]. However, since the data of RIZZOLI[3] have indicated a general decrease of total free amino acid in embryo and yolk during the development, the uptake into new embryo-protein is evidently more rapid than the breakdown of the yolk protein reserves. In 1965, DEUCHAR[34] has formulated the biochemical pattern of transfer of yolk reserve to the embryo as follow: the low molecular weight pool of amino acid appeared in the yolk sac before the reconstruction of the embryonic tissues. It was also suggested from our results that the non-proteic nitrogen did not originate from any particular protein, but from all of three proteins in common. That the yolk proteins of the salmonoid egg have three components has been stated by many investigators[2~7]. Our experimental results and conclusions[1] are similar to those of them. In three components, lipoprotein was dominant, others were glycoprotein and phosphoprotein. We found that the proportion of the three proteins under electrophoretic analysis was unchangable from the start of the development to the later stage. However, since there was an apparent increase of the ratio of nitrogen in HDF after hatching, the breakdown of the lipid-moiety in the yolk protein seemed to be more rapid than that of the protein-moiety. It was also found that the nature of the lipoprotein was gradually altered according to the change of the lipid content. The breakdown of the yolk protein and lipid during the embryogenesis suggested the presence of hydrolases such as proteinase and lipase. The existence of peptidase and esterase in the fish egg have been demonstrated[35,36], but their activities and localization have not yet been investigated. The relation between the appearance of the enzyme activity and the selective consumption of yolk reserves will be subject for further study.

SUMMARY

The analysis of rainbow trout yolk during the development was made on the moisture, lipid, protein, and non-proteic nitrogen concurrently occurred in the yolk sac before consumption by the embryo. The electrophoretic analysis of the protein showed three components distributed there in the ratio of about 83 : 11 : 6, decreasing with the same ratio through all the developmental stages, although a change of the lipid content in the lipoprotein after hatching was observed.

The lipid of lipoprotein rapidly decreased and was consumed preferentially to the oil globule.

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虹鱒卵の生化学的研究

Ⅳ 胚発生過程における卵内容物の変化

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虹鱒卵の未受精期から受精後45日迄の各発生段階における水分，脂質，蛋白質および非蛋白質成分について分析を行った．

卵黄内容物は主として孵化後に大きく変化する．蛋白質は分解され利用されるまで非蛋白態窒素化合物として貯えられる．卵黄蛋白質の電気泳動的観察では，三成分の蛋白質は全発生過程を通じ約83：11：6の割合を示し，著しい変化は認められなかったことから，これらの蛋白質は均等に分解されてゆくと考えられる．

孵化後一時的にリポ蛋白質の脂質部分が蛋白質部分より選択的に分解・利用される時期のあることを認めた．

油球の減少は全発生期間を通じ緩慢であるが，リポ蛋白質の脂質の減少は著しく，油球より利用度の高いことを認めた．