Endocrinological Studies on Lipid Metabolism in Rainbow Trout—I. Differences in Lipid Content of Plasma, Liver and Visceral Adipose Tissue Between Sexually Immature and Mature Females

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Lipids of the liver and the other visceral adipose tissue, as well as plasma of hatchery-reared rainbow trout in different states of ovarian development were compared. Total visceral lipid was decreasing in the maturing female, reduction of triglycerides being most prominent. The content of lipid, especially of triglycerides and free fatty acids in the liver and plasma of the maturing female was increasing. It was suggested from these findings that the stored lipid was moved into the liver, extruded into the blood, and then taken up by the growing oocytes.

Although it is well known that there occurs a remarkable accumulation of lipid within the ova of teleost, mechanism of this phenomenon is only poorly understood. WADA¹⁾, in sardine, and NOMURA²⁾, in rainbow trout, found respectively that the lipid content of liver and the other viscera varied seasonally. These findings may also indicate that the lipid is linked with the physiological process involved in gonadal maturation. These workers, however, did not deal with the blood lipids. Information on the level of blood lipids would seem to be of considerable value in the study of lipid metabolism, because it is clearly demonstrated that the blood plays an important role in transfer of lipid from one part of the body to another in case of mammals. HO and VENSTONE³⁾ reported that the level of plasma lipid in sockeye salmon increased markedly after the estrogen treatment. This result may suggest that blood is closely concerned to the transferring mechanism in teleost.

In the present investigation, the quantitative and qualitative differences of lipid in plasma, liver, and visceral adipose tissue between sexually immature and mature females were analyzed in order to clarify the mechanism of transferring. In mammals, it is well known that the lipid contents of blood and liver are influenced by exogeneous nutrition. Similar phenomenon is suspected to occur in case of teleost. Therefore, the effect of feeding should be checked before the endogenous lipid metabolism is discussed. Ac-

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cordingly, we designed our experiment so that the alteration in lipid levels of liver, visceral adipose tissue, and plasma after feeding might be also elucidated.

Material and Methods

Two-year-old female rainbow trouts, *Salmo irideus*, reared in the pond of Okutama Branch, The Tokyo Metropolitan Fisheries Experiment Station, were used. From fish which had been fed on ordinary trout pellets prepared commercially, seven females at a time were sampled 6 hours, 1, 2, 5, 20, and 85 days after the final feeding.

The samples included sexually immature and maturing fish. At the final sampling, done in January, four individuals were fully matured. Blood was collected after anaesthesia with MS-222 solution, and immediately centrifuged. Liver and the other viscera, which included adipose tissue surrounding alimentary canal, were then dissected out, and stored in a dry ice bath. Separated plasma was also frozen with dry ice. Total lipids of liver and the other viscera were extracted with choroform-methanol mixture (2:1, v/v) by the method of FOLCH *et al.*⁴⁾ Lipid of plasma was prepared by evaporating an aliquot of BLOOR's filtrate.⁵⁾ To determine the changes of lipid constituents, extracts were all analyzed on ascending thin-layer chromatographic plates. The plates were developed with a solvent system of choroform-ether-acetic acid-water (25:10:3:2, by vol.). Another system, hexane-ether-acetic acid (165: 15:1, by vol.) was also employed.⁶⁾ After developments, plates were sprayed with sulfuric acid, and were analyzed densitometrically for quantitative estimation of lipid fractions.

Results

Lipid contents in the plasma, liver and the other viscera of fish which were sacrificed 6 hours, 1, 2, 5, 20, and 85 days after the final feeding are summarized in Table 1. Lipid contents of liver were varied from two per cent to six per cent, although the mean value was 6.35 within a day of feeding, and 4.07 after prolonged starvation. Decrease of

Table 1. Effect of starvation on lipid content in the liver, viscera, and plasma.

Days of starvation	No. of fish	Body weight (g)	Liver			Viscera			Plasma lipid content (g/dl)
			weight (g)	lipid content (%)	(g)	weight (g)	lipid content (%)	(g)	
0	7	256.0	3.46	6.94	0.24	7.92	29.29	2.32	3.80
1	7	288.4	3.91	4.60	0.18	13.22	24.13	3.19	2.54
2	7	235.7	4.45	3.60	0.16	13.02	23.04	3.00	2.68
5	7	271.3	3.87	4.39	0.17	12.07	25.93	3.13	2.17
20	7	277.3	3.83	3.92	0.15	9.90	18.79	1.86	2.01
85	7	243.6	1.66	4.22	0.07	5.97	11.56	0.69	1.54

contents was more conspicuous in absolute amount. The lipid levels in plasma of the fish within a day of feeding were always above three grams per one hundred milliliter of plasma. On the other hand the levels were usually decreased below two grams after prolonged starvation. In viscera, lipid content was about twenty per cent in most cases, and was not altered either by feeding or by short term fasting. However, after severe starvation the amount of visceral lipid was reduced to approximately one half. These results clearly demonstrated that the lipid contents in these organs were modified by the ingestion of food. Accordingly, it was concluded that the trout to be used for the examination of the changes in lipid content as affected by the gonadal maturation should be taken from those sampled after a few days of starvation.

Data of the immature and maturing individuals thus selected are shown in Table 2. Weight of the liver expressed as per cent to the body weight (hepato-somatic index) was larger than that of the immature. Relative amounts of lipid did not differ between these two groups, but the absolute value was larger in the former than in the latter. The amounts of visceral adipose tissue were less in the maturing fish, and so was the lipid contents in both absolute value and percentage. Level of plasma lipid in the maturing female was higher than that of the immature. Differences in the lipid content of liver and viscera were also recognized between fully matured and immature females, which had both been starved for about three months. In starved female the liver was small and its lipid content was extremely decreased. The mean value of the liver weight and hepato-somatic index in matured fish were 1.70 g and 0.6, respectively. These values were not different with those of the immature fish. However, the mean value of lipid content in the liver of the former was 2.12 per cent and was less than that of the latter (6.08 per cent). Moreover, visceral adipose tissue was hardly recognizable and lipid content was reduced in the former. Levels of plasma lipid, on the other hand, were not significantly different.

Table 2. Lipid content of the liver, viscera and plasma of rainbow trout in different states of gonadal maturation.

Gonadal state	No. of fish	Body weight (g)	Gonado-somatic index	Liver				Viscera			Plasma lipid content (g/dl)
				weight (g)	LW/BW	lipid content (%)	lipid content (g)	weight (g)	lipid content (%)	lipid content (g)	
Immature	11	257.0	0.2	3.36	1.3	4.46	0.15	14.43	28.82	4.16	2.37
Maturing	10	284.9	8.1	5.36	1.9	4.48	0.24	11.11	17.64	1.96	2.85
Matured	4	288.6	11.9	1.70	0.6	2.35	0.04	5.70	2.28	0.13	1.44

Lipid constituents as revealed by the thin-layer chromatography are shown in Fig. 1, in which comparisons are made among four different groups, i.e., the immature and maturing fish after a few days' starvation, as well as the immature and matured fish after 85 days' starvation. Lipid constituents of the liver were cholesterol ester, triglycerides,

Fig. 1. Lipid constituents in viscera, liver and plasma of rainbow trout under four different conditions. I: immature female, M: maturing female, I': immature female starved for 85 days, M': fully matured female starved for 85 days.

higher content of free fatty acids and a lower content of phospholipids in the former. As for visceral lipid, although almost all of the constituents were reduced in the maturing female, the decrease of triglycerides was quite distinct. Prolonged starvation, as mentioned above, resulted in a decreased total lipid in viscera. Chromatographically, it was easily recognizable that all constituents were decreased, and that the rate of reduction of triglycerides in fully matured females (M') was more conspicuous than in the immature (I'), in spite of the same duration without food. In case of plasma lipid, the absolute amount of each constituent was increased more or less in maturing female. Especially levels of free fatty acids as well as triglycerides were much higher in both relative and absolute values. After prolonged starvation, despite decrease in the total lipid, larger amount of triglycerides and free fatty acids were still recognized to some extent in case of the sexually advanced females.

Discussion

Seasonal variation of lipid content in the whole body or in the organs of teleost has been reported by several workers. LOVERN⁷⁾ made a study of the seasonal variation of fat content in herring body, and showed that there was a rapid rise in fat content just before spawning season. WADA¹⁾, on the other hand, observed the decrease of the total fat in the liver, muscle, head and adipose tissue in the lean sardine in relation to the gonadal maturation. NOMURA²⁾ reported also that in hatchery-reared rainbow trout the fat in the liver increased in the time of rapid ovarian growth. In the present investigation it was recognized that in maturing female rainbow trout the level of lipid content was higher in the liver and plasma, but lower in the viscera compared to the case of the immature.

free fatty acids, free cholesterol, di- and mono-glycerides, as well as phospholipids (mainly lecithin and cephalin). In case of visceral lipid almost all of the same fractions were encountered, but triglycerides were dominant. Plasma lipid also contained similar constituents, although esterified cholesterol was much more marked than in the liver lipid and visceral one.

Comparison of the lipid fractions of the liver between the maturing fish (M) and the immature (I) indicated a

After prolonged starvation, lipid contents in the liver, viscera and plasma were decreased, although rate of reduction was larger in fully matured female than in the immature. It is reasonable to suppose, therefore, that the gonadal maturation brings about the serious changes in lipid metabolism. The changes of lipid content in the blood with relation to gonadal maturation were known in other oviparous animals. WEXLER and EDMOND⁸⁾ found that the blood of the laying bird contained more fat than that of the non-laying. That the blood of laying hen was characterized by a higher lipid content was also described by WALKER *et al.*⁹⁾ As for variation of constituents, LAWRENCE and RIDDLE¹⁰⁾ discovered a rise in fatty acids and lipid phosphorus in the plasma of laying hen. Furthermore, HEALD and BADMAN¹¹⁾ revealed that in the domestic fowl the free fatty acids increased markedly when the bird came into lay. On the other hand, WEXLER *et al.*⁹⁾ recognized that the blood of the laying hen contained a higher level of triglycerides and free fatty acids. And this phenomenon was interpreted as indication the incorporation into the lipoprotein.^{12,13)}

In this investigation, differences in lipid constituents of plasma, liver and visceral adipose tissue between sexually immature female and the maturing were recognized. In maturing fish, level of free fatty acids and of triglycerides in the liver as well as plasma were higher, while triglycerides in visceral adipose tissue and phospholipids in the liver were reduced. These results, therefore, suggest that the stored lipid was transferred to the liver and then extruded into the blood in form of lipoprotein, and transported to the ovary, where they were taken up by the developing follicles, and laid down in the oocytes as definitive constituents of the yolk.

It can not be assumed, however, that the lipid in the ova has all been originated from the lipid in visceral adipose tissue or in the liver. Because it is well known that the lipids are converted from the proteins or carbohydrates. In fact, glycogen in the liver of rainbow trout varied with gonadal maturation.²⁾ The conversion of these materials and their role in the process of reproduction will be discussed in a separate paper.

References

- 1) S. WADA: Nippon Nogeikagaku Kaishi, **29**, 465-471 (1955).
- 2) M. NOMURA: This Bull., **29**, 976-984 (1963).
- 3) C. F. HO and W. E. VENSTONE: *J. Fish. Res. Bd. Canada*, **18** 858-864 (1961).
- 4) J. FOLCH, M. LEES and G. H. SLOANSTANLEY: *J. Biol. Chem.*, **226**, 497-509 (1957).
- 5) W. R. BLOOR: *ibid.*, **77**, 53-74 (1928).
- 6) E. ARAKI: *The Nissin Igaku*, **50**, 2, 85-92 (1963).
- 7) J. A. LOVERN: *Biochem. J.*, **32**, 676-680 (1938).
- 8) D. E. WEXLER and H. D. EDMOND: *J. Biol. Chem.*, **31**, 281-294 (1917).
- 9) H. A. WALKER, M. W. TAYLOR and W. C. RUSSEL: *Poul. Sci.*, **30**, 525-530 (1951).
- 10) J. V. LAWRENCE and O. RIDDLE: *Amer. J. Physiol.*, **41**, 430 (1916).
- 11) P. J. HEALD and H. G. BADMAN: *Biochim. Biophys. Acta*, **70**, 381-388 (1963).
- 12) W. M. MCINDO: *Biochem. J.*, **72**, 153-159 (1963).
- 13) P. J. HEALD and P. M. McLACHLAN: *ibid.*, **87**, 571-576 (1963).