CCLV. FAT METABOLISM IN FISHES.
IV. MOBILISATION OF DEPÔT FAT IN THE SALMON.

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INTRODUCTION.

THE Atlantic salmon, *Salmo salar*—an anadromous migrant—is from its spawning habits particularly suitable for studies of the mobilisation of depôt fat. The maturing and adult fish feed intensively in the sea, and then, as the spawning time approaches, migrate to the rivers and commence to ascend them. It is certain that from the moment of entering the rivers, if not earlier, the salmon cease feeding entirely. The journey upstream to the spawning grounds demands the expenditure of much energy, which has to be supplied mainly by reserve fat. Moreover, considerable quantities of fat accumulate in the gonads, and this fat is probably transferred from the depôts (*vide infra*). Continuous depletion of the latter thus takes place. Almost all the males perish in the river, presumably from weakness and starvation, but most of the females survive until they reach the sea again. Here most of them presumably die also, as only a small percentage return to spawn a second time. The fish after spawning are known as kelts, and some of these have been found with as little as 0·3 % of fat in the muscles, contrasted with 13–14 % of fat in a fish fresh from the sea.

By selecting salmon of different degrees of emaciation, therefore, it is possible to obtain samples of depôt fat from fish whose percentages of reserve fat form a rapidly decreasing series. By detailed analysis of the fatty acids of these fats it should be possible to show whether mobilisation is a selective process or not. For example, if certain acids, such as the more unsaturated ones, or glycerides containing them, are mobilised more readily than the saturated components, the fats from the more emaciated fish should show an increasing accumulation of saturated components.

EXPERIMENTAL.

The fat was extracted by mincing the whole fish, apart from the viscera, desiccating with anhydrous sodium sulphate and extracting with ether. The viscera (which contain a little fat) were excluded since it was desired only to consider the main depôt fat. Male and female fish were considered separately, in view of possible differences in the extent and type of fat transfer to the gonads. The fatty acid analyses were carried out as described in previous papers. The red pigment of the salmon is an acid substance, soluble in ether, which has been reported on by Euler *et al.* [1933]. It was not possible satisfactorily to remove this from the fat, but it is not likely to constitute a serious source of error in the fatty acid analyses, in view of the relatively small amount present.

The particulars of the fats are given in Table I, and the fatty acid compositions in Tables II and III, as weight and molar percentages respectively.

Table I. *Particulars of salmon depôt fats.*

No.	Sex	Fat content %	Iodine value	Sap. equivalent	Un-saponifiable %	Free fatty acid %
1	♂	13·9	142·4	295·8	0·8	1·0
2	♂	6·5	149·5	299·5	1·3	3·4
3	♂	1·1	161·4	318·8	8·6	22·6
4	♀	13·2	145·0	297·8	0·9	1·0
5	♀	7·3	150·7	302·9	1·3	2·2
6	♀	3·5	139·0	306·8	2·2	7·3

Table II. *Fatty acid compositions (wt. %).*

No.	Saturated C_{14}	C_{16}	C_{18}	Unsaturated C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
1	3·8	15·0	2·0	0·1	10·6	28·8 (−2·8 H)	23·5 (−5·5 H)	16·2 (−6·9 H)
2	2·0	14·1	0·7	—	6·2	27·3 (−2·7 H)	26·9 (−4·7 H)	22·8 (−7·1 H)
3	2·3	13·2	1·0	—	4·3	27·3 (−2·7 H)	25·8 (−5·3 H)	26·1 (−7·8 H)
4	5·0	11·3	1·1	0·5	9·1	25·7 (−2·7 H)	26·5 (−4·7 H)	20·8 (−6·4 H)
5	2·2	12·5	1·6	—	7·7	27·1 (−2·8 H)	28·4 (−4·7 H)	20·5 (−6·8 H)
6	1·9	10·0	1·8	—	7·8	28·5 (−2·6 H)	33·2 (−4·3 H)	16·8 (−6·4 H)

Table III. *Fatty acid compositions (mols. %).*

No.	Saturated C_{14}	C_{16}	C_{18}	Unsaturated C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
1	4·6	16·6	2·0	0·1	11·9	29·2	21·8	13·8
2	2·6	16·0	0·7	—	7·1	28·2	25·5	19·9
3	2·9	15·1	1·0	—	5·0	28·4	24·6	23·0
4	6·3	12·7	1·1	0·6	10·3	26·3	24·8	17·9
5	2·8	14·2	1·6	—	8·8	28·0	26·8	17·8
6	2·4	11·3	1·8	—	8·9	29·4	31·5	14·7

In addition to the above, a sample of salmon ova fat was examined, to determine which acids, if any, were preferentially transferred to the ova. The sample was a mixed one from three fish known locally as "baggots." This term is applied to ripe female fish which have not spawned and are taken early in the year. The weight of eggs to weight of fish varied, as also did the fat contents of the fish and the eggs. It is interesting to note that the fat contents of the fish and eggs form similar series (Table IV).

Table IV. *Particulars of salmon ova.*

Weight of fish g.	Weight of eggs g.	Fat content of fish %	Fat content of eggs %
4656	560	7·3	6·1
6718	993	4·2	5·6
5726	554	3·0	3·3

In the second and third fish the eggs were completely ripe and "running," whilst in the first they were almost so. The mixed fat had an iodine value of

180·9, saponification equivalent 313·6, unsaponifiable 8·0 %, free fatty acid practically nil. The composition of the mixed fatty acids, in weight and molar percentages, is given in Table V.

Table V. *Composition of fatty acids from salmon ova.*

	Saturated			Unsaturated				
	C_{14}	C_{16}	C_{18}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Wt. %	2·3	11·2	1·9	—	12·3	34·4 ($-2·7$H)	21·7 ($-7·2$H)	16·2 ($-9·5$H)
Mols. %	2·9	12·4	1·9	—	13·8	34·8	20·3	13·9

In Tables II and V the mean unsaturation of the C_{18}, C_{20} and C_{22} groups is expressed as lack of hydrogen, as in previous papers.

DISCUSSION.

The salmon fats are of rather peculiar composition. The palmitoleic acid percentage is distinctly lower, whilst the C_{22} acids percentage is higher than usual for most fish fats. The degree of average unsaturation of the C_{20} acids is rather lower than usual. These observations apply to the whole series of salmon body fats and are evidently characteristic of the species and possibly of the Salmonidae family.

In considering Tables II and III, two factors must be borne in mind. Firstly, individual fish will differ appreciably in their fat composition. This is well illustrated by the fact that samples of cod-liver oil, for instance, may vary in iodine value from 160 to 180 or more. Secondly, the analyses are probably not accurate to less than 1–2 units for each percentage. Hence in considering whether or not the data indicate selective mobilisation, account should only be taken of major differences in composition.

For both male and female fish there is a noticeable drop in the proportion of myristic acid as the fat stores are first mobilised, but thereafter the figures remain unchanged. Palmitic and stearic acids remain constant. The content of palmitoleic acid drops steadily in the male series, but it is doubtful how far this is paralleled in the females. C_{18} unsaturated acids remain constant, C_{20} acids constant for the males, with a rise of doubtful significance for the females. C_{22} acids show a marked rise in the starving males but remain constant in the females.

The lack of parallel between the male and female series is possibly due to the different metabolic requirements and processes at spawning time. Another evidence of this is the higher and earlier incidence of mortality amongst the males than amongst the females.

In the male series, it will be observed that the progressive changes which occur tend to exaggerate the specific peculiarities of the salmon fats. This might be considered to indicate a selective process in which the constituents of least value to the fish were preferentially mobilised for use as fuel. Whilst such is a possible explanation, a simpler one can be advanced. In Table VI the figures are given for the total proportions of each group of acids of a particular number of carbon atoms (mols. %).

From this it can be seen that the constituents preferentially mobilised (male series, 1, 2 and 3) are those of low molecular weight, and the least readily mobilised are the high molecular weight C_{22} acids. One can visualise

Table VI. *Acids of same number of carbon atoms (mols. °/₀).*

Fish no.	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
1	4·7	28·5	31·2	21·8	13·8
2	2·6	23·1	28·9	25·5	19·9
3	2·9	20·1	29·4	24·6	23·0
4	6·9	23·0	27·4	24·8	17·9
5	2·8	23·0	29·6	26·8	17·8
6	2·4	20·2	31·2	31·5	14·7

such a selection being brought about by a readier passage of small molecules through the cell membranes—in other words, a permeability effect using the term permeability in a very wide sense. It was shown [Lovern, 1934, 1] that permeability may exercise a controlling influence in the deposition of fat in certain species. The apparent cessation of this process in the case of C_{14} acids when a certain level has been reached can readily be understood. The content of these acids is low to start with. When it falls still lower, mass action effects will counteract the influence of greater permeability to small molecules, and a state of balance will be reached.

One result of this proportionately larger loss of small molecules will be a fall in the proportion of saturated acids. It has been shown earlier [Lovern, 1932; 1934, 1] that such a condition will probably lead to a conversion of palmitoleic acid into palmitic acid. The fall in the palmitoleic acid figures is thus accounted for, and it will be seen that the exaggeration of the species peculiarities may possibly be fortuitous.

It should be noted that considering the large quantities of fat being mobilised, the degree of selectivity exhibited is very low.

In the female series there is only slight evidence of permeability effects, the figures for C_{14} acids being the only ones to show any marked change. The slight fall in palmitoleic acid for the depleted females (Tables II and III) may or may not signify a change into palmitic acid. The significance of the increase in C_{20} acids is very doubtful. It is evident that the degree of selectivity exhibited by the females is even lower than for the males, if indeed it exists at all.

The composition of the ova fat is quite different, however, from that of the depôt fat of the animals, from which it has probably been derived. The proportions of some of the acids, particularly the C_{18}, C_{20} and (to a less extent) palmitoleic acids, are different, and the degrees of unsaturation of the C_{20} and C_{22} acids are considerably higher in the ova fat than in the depôt fat. The writer has observed with other species of fish that the ova fat is more unsaturated than the depôt fat, as shown in Table VII.

Table VII. *Comparison of ova and depôt fats.*

Species	Fat from	Iodine value (of acids)	% Ether-insoluble bromides (from acids)
Cyprinus carpio	Muscles	122·9	Not determined
,, ,,	Liver	152·4	20·8
,, ,,	Ovaries	204·2	37·0
Gadus aeglefinus	Muscles	183·2	37·8
,, ,,	Liver	170·2	Not determined
,, ,,	Ovaries	218·3	53·4
Rutilus rutilus	Muscles	133·4	12·5
,, ,,	Ovaries	159·2	28·8

Lee and Tolle [1934] have examined a number of fats from the livers and eggs of different species of salmon. The egg fats have a higher iodine value than the liver fats in every case.

Channon and El Saby [1932] have shown that in the herring the gonad fat (both male and female) is very highly unsaturated just prior to spawning, even more so than the liver fat. They were unable to ascribe this high unsaturation to selective mobilisation of depôt fat, since there was no steady change in the degree of unsaturation of the latter. So also in the case of the salmon, this selective formation of ova fat cannot be correlated with a change in the remaining depôt fat. It appears that fat is mobilised almost non-selectively in the first instance. If gonad fat is derived from the depôts, as seems most probable, a part of this mobilised depôt fat must be selectively removed by some organ and passed on to the ova, the remainder being consumed as fuel.

There is an alternative explanation of the results, namely that ova fat has not been derived from the depôts, but has been specially synthesised by the fish from carbohydrate or protein sources. Against this idea several facts may be mentioned. The carbohydrate reserves of the fish are not large, and if catabolism for energy requirements is analogous in the salmon to that of mammals, this carbohydrate must be largely consumed along with the fat, or ketosis would result. Also, the production of fat from protein would be such a wasteful process that it is almost unthinkable in the case of a starving animal, especially in view of the large amounts of special proteins deposited in the developing gonads. Lastly, in the case of mammals there is increasing evidence that fat synthesised from carbohydrate is of a relatively simple type, and it may be that the same applies to fishes.

It is somewhat unfortunate that the ova concerned were obtained from "baggots." The ova in two of these fish were matured in the sea (as shown by the scales) and there is thus a strong possibility that the fish were feeding whilst the ova matured. The writer intends to examine the fat from both male and female gonads which have beyond question been matured in the river and hence in starving fish.

The selective transfer of very highly unsaturated constituents to the ovaries has a possible parallel in the selective acquisition by the phosphatides of certain animals of the most unsaturated of the available acids [Sinclair, 1932].

The high degree of unsaturation of the egg fat is not evidence of desaturation, since the C_{20} and C_{22} acids consist of mixtures of monoethylenic acids with a tetraethylenic acid in the case of the C_{20} and a pentaethylenic acid in the case of the C_{22}. Still other acids may well be present. The unsaturation in the ova fat does not reach that of tetra- and penta-ethylenic acids for the C_{20} and C_{22} acids respectively. Hence the observed increase in average unsaturation is quite compatible with a selective transfer of the required proportions of the different acids or glycerides to the ovaries, without any process of desaturation. The significance of the ova fat composition is dealt with further in the following paper [Lovern, 1934, 2].

Quagliariello [1933] has adduced evidence in the case of the dog that when depôt fat is mobilised it is first hydrolysed by lipase followed by desaturation before leaving the depôt cells. The increasing content of free fatty acid in the salmon depôt fats as the depôts become depleted (Table I) is of interest in this respect although alternative explanations are possible. In general, too, the iodine values increase as the depôts are depleted, as found by Quagliariello, but salmon 6 is a noteworthy exception. All the salmon were fairly fresh when extracted and about equally so.

It may be that lipase was more active in the emaciated fish, giving a greater *post mortem* production of free fatty acid. On the other hand, it might be that in the living animal there was an appreciable percentage of free acid in the starving fish. If so, its accumulation as fat is removed might be analogous to that of the unsaponifiable matter (mainly cholesterol); presumably a simple concentration. Channon and El Saby [1932] observed a similar increase in the percentage of unsaponifiable matter as the depôts were depleted in the herring. It is doubtful how far the results support the theory of Quagliariello.

SUMMARY.

Determinations have been made of the fatty acid compositions of the depôt fats from a number of salmon in various states of starvation. The data seem to indicate an almost non-selective mobilisation of the depôt fat, or at most a very low degree of selectivity, for the females, but some selectivity in the case of the males.

If fat is transferred to the developing ovaries, a high degree of selectivity is displayed, and this must be exercised by some organ. The remainder of the fat mobilised, which is not transferred to the ovaries, is presumably used as fuel.

The salmon depôt fats all show several peculiarities of composition, probably characteristic of the species.

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