IV. FAT METABOLISM IN FISHES.

VIII. CHANGES IN THE FAT OF RIPENING SALMON EGGS.

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IN a previous paper [Lovern, 1934, 2] it has been shown that the fat of ripe salmon eggs is different in type from salmon body fat. Apart from higher average unsaturation of the C_{20} and C_{22} acid groups, the content of C_{18} acids is distinctly higher. The egg fat indeed suggests a fat of the fresh-water type, with the specific peculiarities of the salmon superimposed upon it. It was further shown that whilst this egg fat must have been deposited selectively, the selection was not exercised by any specific withdrawal from the depots. The work now to be described is a closer study of the mechanism of this selective deposition of egg fat.

EXPERIMENTAL.

Samples of salmon ova were collected in various stages of maturity. The least developed were obtained from "fresh run" fish in February and March. Intermediate samples were obtained from fish taken up river in May, July and September. Fully ripe ova were obtained from fish actually spawning in the Tweed during November of 1934. In order of increasing development these will be referred to as samples Nos. 1 to 5. Sample No. 1 represented about 100 fish, the others considerably fewer. No. 3 was from one fish only. Apart from No. 3, however, sufficient fat was available in each case for analysis by full-scale methods. The fat contents of the ova were found to be irregular, but of the same order throughout. They ranged from 7 to 15 %. The fats were freed from phosphatides, and their characteristics are given in Table I. The most significant variation is the steady fall in iodine value with ripening.

Table I. *Particulars of salmon ova fats.*

No.	Iodine value	Sap. equivalent	Unsaponifiable %
1	206.1	315.9	8.8
2	198.2	320.6	7.4
3	194.3	327.1	9.0
4	174.8	315.9	6.3
5	176.1	311.9	7.2

The compositions of the mixed fatty acids (as wt. %) are given in Table II. In the case of No. 3 which had to be examined on a semimicro-scale, a series of accidents occurred. These rendered it impossible to obtain a reliable complete analysis for this fat, but it was possible to calculate the content of C_{18} unsaturated acids and the degree of average unsaturation of these. In Table II are included

for comparison the results on the ripe ova fat from "baggots"¹ recorded previously [Lovern, 1934, 2].

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

No.	Saturated			Unsaturated				
	C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂
1	3.1	16.0	0.5	0.1	12.6 (-2.0H)	23.7 (-4.0H)	27.2 (-8.0H)	16.8 (-10.4H)
2	2.9	13.4	0.7	0.4	10.4 (-2.0H)	25.3 (-3.2H)	28.7 (-7.2H)	18.1 (-11.4H)
3	—	—	—	—	—	28.0 (-3.1H)	—	—
4	1.8	13.0	2.0	—	9.9 (-2.0H)	36.7 (-2.9H)	21.3 (-7.6H)	15.3 (-11.1H)
5	2.3	12.9	2.2	—	9.6 (-2.0H)	34.8 (-2.7H)	23.2 (-7.6H)	15.0 (-11.2H)
Baggot	2.3	11.2	1.9	—	12.3 (-2.0H)	34.4 (-2.7H)	21.7 (-7.2H)	16.2 (-9.5H)

DISCUSSION.

Table II reveals several points of interest. (a) It will be seen that although "baggots" are abnormal fish as regards spawning, yet the egg fat is not significantly different from that of normal ripe fish (No. 5). (b) The high degree of unsaturation of the C₂₀ and C₂₂ groups is in evidence throughout with no progressive or drastic changes in value. (The corresponding degrees of unsaturation for salmon body fat are about -5 H and -7 H.) (c) As ripening progresses there is a fall in the percentages of C₁₄, C₁₆ and C₂₀ acids, C₂₂ acids remaining practically constant. (d) There is a marked rise in the content of C₁₈ unsaturated acids and a small but significant rise in stearic acid content with development. (e) The degree of unsaturation of the C₁₈ acids steadily decreases from No. 1 to No. 5.

The composition of sample No. 1 is noteworthy in that the percentages of the various acid groups are not significantly different from those of a salmon body fat [Lovern, 1934, 2]. It differs markedly as regards the degrees of unsaturation of the C₁₈, C₂₀ and C₂₂ acids. It was evident during analysis that monoethylenic C₂₀ and C₂₂ acids were absent from all the egg fats. In the body fats they were present in considerable amount. Oleic acid also appeared to be almost absent from sample No. 1. At least two interpretations of these facts are possible: (1) That depot fat is taken, the monoethylenic acids (except palmitoleic acid) selectively removed, and the rest passed on into the ova. (2) That the C₁₈, C₂₀ and C₂₂ monoethylenic acids are desaturated either before the fat is deposited, or else in the ova themselves. Theory (1) is less likely, since it would, in its simplest form, lead to greatly diminished quantities of C₁₈, and especially of C₂₀ and C₂₂, acids, together with a very high content of C₁₄ and C₁₆ acids in the ova. The C₂₂ acids of the ova fat consisted largely of an acid with 6 double bonds. The percentage of such an acid, if any, present in the depot fat, must have been very low. If this acid were selected from the mixed C₂₂ acids available, whereas the C₁₄ and C₁₆ acids were taken in their entirety, the ratio of C₂₂ to C₁₄ or C₁₆ acids in the ova fat would be very much lower than is the case. It is also difficult to picture a mechanism which could remove these three monoethylenic acids so selectively. Whilst not impossible, it is very unlikely that there is any peculiarity in the

¹ "Baggot" is a term applied to ripe female salmon taken early in the year which, for some reason, have not spawned.

glyceride structure of these oils that could lead to certain glycerides being selected containing amongst them all the acids with the exception of monoethylenic C_{18} , C_{20} and C_{22} acids. Theory (2) would account for the observed composition of the egg fats. It has been shown [Lovern, 1935] that fish are probably able to synthesise polyunsaturated acids of 20 and 22 carbon atoms and a desaturation mechanism may possibly be common. It is the rule for fish egg fats to be more unsaturated than the depots from which they are drawn [Lovern, 1934, 2].

Turning to sample No. 2, the greatest change which has occurred since the No. 1 stage is the marked fall in degree of unsaturation of the C_{18} acids. Once again there are two possible explanations: (A) that as development proceeds the desaturation mechanism (or other means of eliminating monoethylenic acids) is confined to the C_{20} and C_{22} acids only, C_{18} going in with about -2.7 H average unsaturation; (B) that actual hydrogenation of the C_{18} acids is taking place. There is no evidence to decide between these theories.

This fall in unsaturation of the C_{18} acids continues throughout the whole series of fats, and there is moreover a steady increase in the content of C_{18} acids. It is obvious, however, that addition of less unsaturated C_{18} acids to a fat of the composition of No. 1 would not in itself account for the observed fall in unsaturation of the total C_{18} group. Considering only Nos. 1 and 2, the added C_{18} to raise the percentage from 23.7 to 25.3 and yet lower the unsaturation from -4 H to -3.2 H would have to have a negative iodine value. Extra C_{18} acids are undoubtedly added to the fat, but in addition either theory (A) or theory (B) above must apply.

The origin of these extra C_{18} acids is obscure. The weight of a roe in sample No. 5 was about 800 g. An average figure for the fat content is 10%. Hence the ova finally contain about 80 g. of fat of composition No. 5. Of this about 8 g. must be "added" C_{18} acids. This must either have come from the depots or have been synthesised from non-fatty sources. It is suggestive that the greatest increase in the C_{18} percentage comes after the fish has been up river for some time. Its state of starvation is already well advanced. In this condition it is unlikely that much non-fatty matter could be spared for fat synthesis, especially when so much depot fat is already available. It was shown previously [Lovern, 1934, 2] that there is no evidence of any selective withdrawal of C_{18} acids from the depots. Such a selection would have to be made by some membrane or organ after the fat had left the depot. It is hard to imagine it taking place from a mixture of glycerides, since simple tri- C_{18} glycerides would not be expected in great amount. If C_{18} acids were picked out from the total acids available in the mobilised depot fat and transferred to the developing ova, then some stearic acid would accompany them. The observed rise in stearic acid from samples Nos. 1 to 5 is in accord with this. Separation of acids according to molecular size has been suggested before [Lovern, 1934, 1].

The addition of extra C_{18} acids to the total should result in a fall in the percentages of all other components. This is the case except for C_{22} acids which remain approximately constant. It has been pointed out previously [Lovern, 1934, 2] that one of the specific salmon peculiarities is a higher C_{22} percentage than usual. That specific properties are deep-rooted is certain. It seems of importance that the salmon egg fat should be a salmon fat, even though having other characteristics. To maintain this almost constant C_{22} acid content in spite of added C_{18} acids, necessarily involves a slightly preferential transfer of C_{22} acids from the depots to the ova.

In the previous work on the mobilisation of salmon depot fat [Lovern, 1934, 2] it was shown that for male salmon, selectivity took the form of a more ready

mobilisation of small molecules. This led to a rise in C_{22} acids in the depots of the starving fish. In the case of the females, the same selectivity appeared but was not so evident, in that C_{22} acids decreased instead of increasing. The reason for this can now be suggested. Male salmon gonads at all stages of development contain less than 1% of fat, so that even if this fat is formed selectively it can have very little effect on the depots. For the females, however, there is now evidence of a preferential withdrawal of C_{22} acids. This must be in spite of the readier mobilisation of small molecules and must be controlled by a different mechanism. Any attempt to calculate the quantity of extra C_{22} acids mobilised and correlate this with the observed changes in the depots would be useless because the depots examined were those of a single fish in each case. Individual peculiarities, together with experimental errors, might lead to wide discrepancies. This theory can only be suggested qualitatively. As in the case of the C_{18} acids, it is difficult to imagine such a mechanism functioning with glycerides, but simpler with acids. In the present case, in contrast to the C_{18} acids, the operation affects the composition of the remaining depot fat and therefore takes place in the depots. There is, moreover, evidence that hydrolysis in the depots is the first stage of mobilisation. This was alluded to in the previous paper [Lovern, 1934, 2] and the marked rise in free acid in the depots of starved fish deserves further investigation. It would be of great interest to determine whether this free acid consisted mainly of the higher acids, as it should do on the theory of hydrolysis followed by preferential removal of the lower acids.

A further point must needs remain unsettled until methods have been perfected for dealing with it. It would be of interest to know whether the added C_{18} acids have been incorporated as a simple triglyceride or have been added to the other acids before converting into mixed glycerides. If the latter, it is further evidence that the fat was mobilised in the form of acids.

Finally, reference must be made to the work of Channon and Saby [1932] on herring ova fat. They found a steady rise in the iodine value of the fat as the ova developed. Whilst iodine values alone are not a reliable guide to what is happening in a fat, this rise would not be expected in view of the salmon results, wherein, apart from the C_{18} peculiarities (which may well be concerned primarily with the fact that salmon eggs are to be laid in fresh-water) there is no reason for the mean iodine value of the whole fat to change appreciably. Experiments have accordingly been made with herring ova in various stages of ripening. Several hundred were taken in each sample. Care was taken to remove contaminating body fat which is always present at certain stages of development when the herring flesh is particularly rich in loosely-held fat. Phosphatides were removed although the amounts were only small. Channon and Saby did not make this separation. The fats were found to have almost the same iodine values throughout, with no evidence of a rise during development.

SUMMARY.

Analyses have been made of the fat of salmon eggs at different stages of maturity. At all stages the degrees of unsaturation of the C_{20} and C_{22} acids are higher than in the depot fat and two possible explanations of this are discussed.

With ripening there is a marked rise in the proportion of C_{18} acids together with a fall in the mean unsaturation of this group. There is as yet insufficient evidence to indicate the mechanism of these changes. The result is that the ova fat finally takes on some of the characteristics of a fresh-water fish fat, whilst still retaining the specific peculiarities of a salmon fat.

There is evidence of a slightly preferential withdrawal of C_{22} acids from the depots and this agrees with observed changes in the depots of starving fish. Male salmon gonads are relatively non-fatty and the different requirements of the sexes during spawning are reflected in the compositions of the depot fats. In contrast to the C_{22} acids, the preferential translocation of C_{18} acids to the ova does not affect the depot fat composition, and the selective process must operate after the fat has been mobilised.

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