86. FAT METABOLISM IN FISHES

14. THE UTILIZATION OF THE ETHYL ESTERS OF FATTY ACIDS BY THE EEL AND THEIR EFFECT ON DEPOT FAT COMPOSITION

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IN previous papers in this series [e.g. Lovern, 1937] the theory has been advanced that certain fish have the ability to hydrogenate or dehydrogenate their depot fats according to their specific requirements in relation to the type of fat ingested. Similar metabolic processes have been suggested for various mammals [Banks & Hilditch, 1931; 1932; Hilditch, 1937; Schoenheimer & Rittenberg, 1936; Rittenberg & Schoenheimer, 1937; Longenecker, 1939]. The theory has yet to be verified, however, by feeding an individual fatty acid at a high level and observing an increase in the corresponding saturated or unsaturated acid.

Using eels as experimental animals, an attempt has been made to obtain such evidence by feeding certain ethyl esters. The acids chosen were myristic, palmitic and the mixed unsaturated acids of eel fat. Ingestion of either of the first two acids in quantity should raise the content of saturated acids in the eel's depot fat. If the theory is correct and applies to the eel, this should lead, in turn, to a compensatory dehydrogenation of some of the saturated acid, and since tetradecenoic acid is not normally an important constituent of fish fats, the probability would be that palmitic acid would be dehydrogenated. Thus feeding of either ethyl myristate or palmitate might be expected to raise the content of hexadecenoic acid in the eel's depot fat. Conversely, feeding of unsaturated esters should lead to a compensatory hydrogenation process, and since there would be little tetradecenoic acid available for this and as stearic acid (and higher saturated acids) are not usually present in other than very small quantities in fish fats, the expectation would be that hydrogenation of hexadecenoic acid would be the most important process. Such changes, if of sufficient magnitude, should be detectable by comparing the fatty acid compositions of the fats from the experimental eels and normal eels.

EXPERIMENTAL

In the first experiments batches of 35 eels of 30–35 cm. in length were confined in large tanks supplied with slowly-running water. No temperature control was incorporated as the experiment was made during the summer when feeding should be satisfactory at the prevailing temperature. Attempts to feed the esters directly by pipette were unsatisfactory owing to subsequent vomiting. Accordingly the esters were injected subcutaneously into earthworms (killed by freezing) and such prepared worms were readily swallowed by the eels. If too large a quantity of an ester was injected into a worm at the start of the experiment, such a worm led to subsequent vomiting, but by gradually increasing the

amount the eels in the course of about a week were able to take as much ester as the worms could contain (up to 1 ml. per worm), without apparent digestive disturbances. The number of worms eaten per tank per day was noted, from which an approximate idea of the ester consumption was obtained. The ethyl myristate and palmitate were mixed with 15 and 20 % respectively of eel fat, to furnish on digestion a mixture of acids rather than a single acid, since the work of Cox [1933] suggests that better absorption is thereby possible.

Ethyl palmitate and the unsaturated esters were readily acceptable to the eels (treated worms being taken almost as readily as untreated ones), but ethyl myristate was not well received and consumption throughout the experiment was very poor and erratic.

After a few weeks of fairly satisfactory feeding, the daily consumption began to decline, possibly owing to falling water temperatures. Feeding was continued, however, until all the prepared esters had been given when the fish were killed and the body fats extracted and analysed. The results are given in Tables 1 and 2.

Table 1. *Details of feeding*

1st Series (35 eels per tank)			
Tank no.	...	1 (Myristate)	2 (Palmitate) 3 (Unsatd. esters)
Initial wt. eels (g.)		3247	2757 2984
Final wt. eels (g.)		2411	2019 2326
		Loss 836	Loss 738 Loss 658
Duration of experiment (weeks)		14	14 14
Esters eaten (ml.)		95	178 234
Final fat content of eels (%)		17.4	13.1 14.7
2nd Series (35 eels per tank)			
Initial wt. eels (g.)		—	1952 1959
Final wt. eels (g.)		—	1958 1814
			Gain 6 Loss 145
Duration of experiment (weeks)		—	11 8.5
Esters eaten (ml.)		—	312 243
Final fat content of eels (%)		—	8.8 8.9

Table 2. *Composition of fatty acids of eel body fats (%)*

Material	Saturated			Unsaturated				
	C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂
Untreated eels	4.3	17.3	2.1	0.1	9.0 (-2.2 H)	28.9 (-2.6 H)	20.4 (-5.8 H)	7.9 (-9.7 H)
Fed myristate	5.5	17.3	2.2	1.3	7.6 (-2.2 H)	35.8 (-2.7 H)	18.9 (-5.8 H)	11.4 (-10.0 H)
Fed palmitate (1)	5.5	18.7	1.5	0.7	8.8 (-2.1 H)	36.9 (-2.6 H)	17.9 (-5.7 H)	10.0 (-9.2 H)
Fed palmitate (2)	5.8	25.7	2.2	0.4	9.1 (-2.2 H)	37.1 (-2.5 H)	13.6 (-5.6 H)	6.1 (-8.5 H)
Fed unsaturated esters (1)	5.4	17.8	1.4	0.7	7.1 (-2.1 H)	39.3 (-2.8 H)	18.2 (-6.1 H)	10.1 (-9.3 H)
Fed unsaturated esters (2)	5.4	16.0	2.1	—	6.0 (-2.2 H)	42.6 (-2.6 H)	20.9 (-5.2 H)	7.0 (-10.5 H)

In view of the losses in weight during the experiment and the failure appreciably to modify the fatty acid composition, a second series of experiments was undertaken the following summer, using an elevated and controlled water temperature (20°), which has already been shown to increase feeding intensity

[Lovern, 1938]. This led to a much more regular and higher food consumption, but once again ethyl myristate proved unacceptable and its feeding was discontinued. The results of these later experiments are also given in Tables 1 and 2. The figures for ester consumption have been corrected for admixed eel fat. The worms themselves contained less than 2% of fat.

DISCUSSION

The loss in weight of all the eels in the first experiments is in keeping with the results of earlier work [Lovern, 1938] where it was found that captive eels only gained in weight when the calorie intake was considerable. A curious feature of these eels is the high content of fat (%) at the end of the experiment. From their length they should have started with about 9–10% of fat [Lovern, 1938], and, in fact, a sample tested gave such a figure. These eels were also unusually thick for their size, and this is reflected in the higher weights of 35 fish in exp. 1 than in exp. 2, where the eels were also roughly of the same length. From all the feeding experiments on eels which the writer has carried out, it would appear that these fish do not necessarily utilize primarily fat during partial starvation, but may use protein instead, thus actually having a higher fat percentage after loss of weight. (This seems to apply especially in cold water.)

In the second experiments there was no great change in total weight and the fat content was normal for eels of that size.

From Table 2 it can be seen that whilst in exp. 1 the results have been in general what would be expected if the theory of interchangeability is correct, the extent of the changes is, in general, too small to be outside experimental error. On the myristate diet the most notable effect has been an increase in tetradecenoic acid, which, although small, is probably outside experimental error. The composition given for untreated eels is an average of two analyses for eels of 30–35 and 50–55 cm. long respectively [see Lovern, 1938]. These analyses agreed closely and from all other eel fat results given in this earlier paper it would seem that a figure of 0.1% of tetradecenoic acid is not likely to be greatly exceeded. The contents of myristic and tetradecenoic (with one exception) acids are apparently higher than normal in all the fats from eels fed on earthworms and this may be due to ingestion and deposition of small amounts of lauric and dodecenoic acids derived from the worm fat [see following paper]. Traces of such acids would not be detectable as such in the eel fats, but would result in apparently higher contents of C_{14} acids at the expense of the C_{16} acids. The only previous eel fats encountered with more than about 4.5% of myristic acid were from fish fed on herring, the fat of which contained a relatively high content of myristic acid [Lovern, 1938]. If the abnormally high content of tetradecenoic acid in the fat from eels fed with myristate has any significance, it suggests a desaturation process during absorption in this case, rather than after deposition, since the stimulus of an abnormally high content of saturated acids in the depot fat has not been effected.

On the palmitate diet in exp. 1 there has been a slight rise in the palmitic acid figure, but no corresponding rise in hexadecenoic acid. The fat from the eels fed on unsaturated esters has a rather low content of hexadecenoic acid, whereas it should have tended to rise slightly if no hydrogenation had occurred. The unsaturated esters fed had the following percentage fatty acid composition: sat. acids, C_{14} , 2.4; C_{16} , 5.7; unsat. acids, C_{14} , 0.5; C_{16} , 11.5 (–2.3 H); C_{18} , 45.4 (–2.8 H); C_{20} , 25.8 (–5.9 H); C_{22} , 8.7 (–9.2 H). However, once again the stimulus of an abnormally low content of saturated acids in the depot fat is

lacking, and if hydrogenation of hexadecenoic acid has occurred it has probably been during the absorption processes.

Turning to the second series of experiments, the results have been in the same direction, but more definite. On the palmitate diet the content of palmitic acid has risen by 8.4 units, a figure far beyond experimental error and undoubtedly due to assimilation of palmitic acid from the ethyl palmitate. It is noteworthy that this should be possible with a cold-blooded animal when the ester itself is solid at the water temperature (even when mixed with 20 % of eel fat) and the acid, of course, would be quite hard. There was no visible evidence of bad absorption of the acid, no large fatty stools being observed, such as Cox [1933] noticed with rats fed with ethyl palmitate or stearate. It is clear from Table 1 that the amount of palmitic acid finally appearing in the depot fat is only a small fraction of that fed, and this result is to be expected by analogy with earlier work on eels fed on herring [Lovern, 1938]. The remainder of the ingested fat is presumably utilized for energy purposes, and it is notable that these eels were the only ones to maintain their weight throughout the experiment.

The incorporation of this extra acid in the depot fat will, other things being equal, cause a fall in the proportions of all other constituents and the figure for hexadecenoic acid in the untreated eel fat would fall to 8.1 % if sufficient palmitic acid was incorporated to raise the total palmitic acid content to 25.7 %. If the total saturated acid content of the fat is considered, rather than palmitic acid alone (it has been indicated that the apparent rise in myristic acid is at the expense of palmitic acid, and in any case it is the total content of saturated acids which would provide the stimulus to dehydrogenation in the depot fat), the corrected figure for the hexadecenoic acid content of the untreated eel fat becomes 7.8 %. Obviously the content of all the other unsaturated acid groups would also fall. Actually, however, the hexadecenoic figure has not fallen but the content of the other unsaturated acids has fallen, as would be expected.

The results therefore are just as would be predicted by the theory of dehydrogenation of palmitic acid under suitable stimulus, but the small extent of the changes makes their evidence uncertain. A difference between 9.1 and 7.8 % of hexadecenoic acid is probably just outside the limit of error to be expected by an experienced worker, but there remains the question of normal variation in eel fat composition. Only two normal eel fat analyses are available, and, as already mentioned, the agreement there was close. Each analysis represented many fish but there seems no reason why small variations should not be expected from season to season, owing to differences in food supply, etc. If all the fats from eels fed on fat-poor diets are considered [Lovern, 1938], quite considerable variations are shown, but the average figure for hexadecenoic acid for all these samples is below 9 % and it seems unlikely that this figure should be exceeded in the fat from normal eels taken from these particular waters (the estuary of the River Dee). The one sample which did have a higher content of this acid was abnormal in other respects (high content of palmitic acid and unusual content of fat in the fish) suggesting that this batch of fish had had an unusual dietary history. Nevertheless, in view of the combined experimental error and natural variation, it would be unsafe to regard the results as a proof of the theory, although they are absolutely in accord with it.

Turning to the second experiment in the feeding of mixed unsaturated esters, such a diet should lower the content of palmitic and, to a very slight extent, raise that of hexadecenoic acid. In effect a slight fall in palmitic acid has taken place (the figure of 16.0 % being the lowest ever encountered in any eel fat,

normal or otherwise) and the hexadecenoic acid figure, instead of rising, has also fallen.

These results are also just as would be predicted on the theory of hydrogenation of hexadecenoic acid under suitable stimulus, part of the ingested acid being hydrogenated (either during absorption or in the depots) in an attempt to maintain the normal content of saturated acids. The apparent entire absence of tetradecenoic acid is also of interest and again suggests a hydrogenation process. However, the previous remarks regarding combined experimental error and natural variation apply here also.

Considering all the results of both series of experiments it may be said that they are all in accordance with the theory and also that the extent of the changes has varied in the appropriate way with the intensity of feeding. It seems rather unlikely that experimental error and natural variation have in all cases caused such a situation and the writer believes that whilst more certain data are highly desirable, these results do provide additional support for the theory of interconvertibility of certain saturated and unsaturated acids.

SUMMARY

In two series of feeding experiments, ethyl myristate, ethyl palmitate and the ethyl esters of the mixed unsaturated acids of eel fat have been fed to eels. Rather surprisingly, good absorption on the palmitate diet was indicated.

The diets produced certain modifications in the composition of the eel body fats, but apart from a noteworthy increase in the content of palmitic acid when ethyl palmitate was fed, the changes were of too small an order to be differentiated with certainty from combined experimental error and natural variation.

The consistency of these changes, however, increases the probability that they were genuine. Feeding ethyl myristate caused a rise in tetradecenoic acid, ethyl palmitate caused a rise in hexadecenoic acid and the mixed unsaturated esters gave results suggesting hydrogenation of hexadecenoic acid.

The results, therefore, whilst not conclusive, afford additional support for the theory of the interconvertibility by fish of certain saturated and unsaturated acids.

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