CLXIII. FAT METABOLISM IN FISHES

XIII. FACTORS INFLUENCING THE COMPOSITION OF THE DEPOT FAT OF FISHES

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THERE are at least four factors which possibly affect the composition of the depot fat of a fish. These are (1) species, (2) diet, (3) and (4) temperature and salinity of the water.

Species. It is well known that different species of animals lay down depot fats more or less characteristic of that animal. With normal diet the composition of the depot fat will remain roughly the same. In the case of fish the author [Lovern, 1937] has shown that various characteristic depot fats are produced from a roughly uniform diet, according to species, their composition thus being largely independent of diet.

Diet. It is equally well known that the diet of an animal may affect the composition of its depot fat. The process, however, is by no means quantitative, and when it is studied in detail some contradictory results are obtained. There are two types of experiment which throw light on this problem.

In the first type certain fatty acids are fed which can be detected if they are incorporated in the depot fat, e.g. elaidic acid and acids containing deuterium. Schoenheimer & Rittenberg [1935] found that in mice fed for 4 days on a diet containing 1 % of fat containing deuterium (the only fat in the diet), 47 % of the ingested fat was found in the depots. The authors conclude that the largest part of the dietary fat, even at this low level of ingestion, is deposited in the fatty tissues before it is used. In another experiment the same workers [Rittenberg & Schoenheimer, 1937] find evidence of a continual turnover between depot fat and ingested fat and suggest a half-lifetime for depot fatty acids of 5-9 days.

In the second type of experiment fats are fed which differ from the normal depot fat of the animal in the proportions of the different acids present, or in the unsaturation of some of these. Here the results obtained are quite different and sometimes contradictory. Thus, Thomas *et al.* [1934] found that liberal allowances of whole soybeans, menhaden oil, corn oil and coconut oil fed to steer calves for 260 days had no perceptible effect on the unsaturation of the body fats. On the other hand, Ellis [1933] found a marked decrease in saturation in pig's fat after feeding peanuts and an increase in saturation after feeding corn. Possibly the different species of animal used has something to do with this apparent contradiction. It should also be remembered that whilst a change in iodine value indicates a change in composition, a constant iodine value does not necessarily connote a constant composition.

The experiments of this second type uniformly indicate that fat must be fed at a fairly high level before any considerable effect is produced on the body fat. Thus, Banks & Hilditch [1932] found 1-2 % of C_{20} and C_{22} unsaturated acids in the fat of a pig fed on a diet containing 0.7 % of fish fat. Brown [1931] fed pigs on a diet containing 14 % of menhaden oil, but the content of C_{20} and C_{22}

unsaturated acids in the fat was only 2.7%, so that all this extra fish fat had had little effect on the depot fat. Bhattacharya & Hilditch [1931] showed that 3% of shea butter or of arachis oil in the diet of a pig made little difference to the proportions of the various acid groups, and suggested that acids ingested in excess are disposed of (by oxidation) to leave a residue "more or less adapted to the requirements of the particular storage fat". Again, Banks *et al.* [1933] found that when about 5% of cod liver oil was included in the diet of rats, there was not much alteration in the depot fat, apart from some deposition (about 8%) of C_{20} and C_{22} acids. The rest of the acids appeared to be present in much the same relative proportions as when the diet was mainly fat-free. Therefore the ingested cod liver oil had not been freely incorporated in the rat's depot fat.

The discrepancy between the results obtained by experiments of these two types may be understandable. There are two characteristics of a fatty acid which may affect a fat in which it is incorporated—unsaturation and length of carbon chain. It may be that the requirements of an animal with regard to the chain lengths of its depot fatty acids are specific. In the case of fish, where there is a great range of chain lengths amongst the various fatty acids, the relative proportions of these groups of acids are often quite characteristic of the species. Now a fatty acid containing deuterium, for instance, might well become incorporated in the depots in place of some normal acid of the same chain length without upsetting the animal's specific requirements. Whatever the reason, there seems little doubt that whilst normally there is constant interchange between ingested fat and depot fat (as shown by the deuterium experiments), this is not unconditionally the case when a fat with unusual proportions of the various acids is fed in the diet. Some selective mechanism then operates, and surplus ingested acids of various chain lengths are eliminated or modified until a mixture resembling the normal depot fat remains. If, however, the unusual fat fed is above a certain limit this selective mechanism partly breaks down.

There has been very little work done on the effect of different dietary fats on fish.

Temperature. It is generally accepted that temperature has an effect on the composition of the fats of both animals and plants, higher temperatures tending to produce more saturated fats. It should be noted that this only applies to a given species under varying conditions of temperature. This effect has been studied, for example, in pigs, and Dean & Hilditch [1933] have confirmed and amplified the earlier results of Henriques & Hansen [1901]. In the case of fish, Brocklesby & Bailey [1932] found an increasing iodine value in the body oil of the salmon as they went further north (provided that they kept to the same species of salmon), and stated that this corroborated the findings of investigators working on other species of fish. Tropical fish do not necessarily have a more saturated fat than other (even closely related) species inhabiting colder waters [Lovern, 1932; 1935, 2].

Salinity. It has been shown repeatedly [Lovern, 1937] that fresh-water fish have fat different from that of marine fish, but the reason has not yet been found. The difference is also found in the vegetable-feeding zooplankton [Lovern, 1935, 1]. These small Crustacea must produce these characteristic types of fat by appropriate modification of ingested fat, at least in the case of the marine organisms [Lovern, 1936]. Whilst this effect is apparently associated with salinity alone, it may be an indirect effect. Thus, Fox & Simmonds [1932] have found that the oxygen uptake of Crustacea is greater in fresh water than in salt water, and the whole metabolic rate is higher.

In the experiments described in this paper an attempt has been made to study the individual effects of diet, temperature and salinity on the fat of a suitable species of fish. The common eel, *Anguilla vulgaris*, was chosen because (a) it has a high fat content and adequate quantities of material can be obtained from a reasonable number of individuals, and (b) it can live in fresh water or sea water.

EXPERIMENTAL

The apparatus consisted of three large tanks, each of which was connected to a smaller tank. The small tanks, placed below the larger ones, were fitted up as thermostats with both heating and cooling appliances. Water ran continually through gravel filters from the upper into the lower tanks, and was pumped back as an aerated froth. The eels were confined in the large tanks. They were fed daily, and each day the water was changed, affording an opportunity for cleaning the tanks and removing unconsumed food. Refilling was done slowly through the thermostatic tanks to minimize temperature disturbances.

In the first experiment the tanks were set up as follows: 1, warm sea water; 2, warm fresh water; 3, cold fresh water. Each tank was stocked with 50 eels 30–35 cm. in length. Until feeding had commenced the temperatures were maintained at about 15°. In fresh water the eels refused food for about 10 days, but in sea water they commenced feeding at once. The diet consisted of shelled mussels (*Mytilus edulis*) and was fed *ad lib.* After feeding had become general, the temperatures were adjusted to the highest and lowest limits at which feeding was satisfactory. On raising the temperature progressively from 15°, the daily consumption of food increased until a temperature of 23° was attained. After this point consumption declined, and so this temperature was maintained for the two warm-water tanks. Similarly, on lowering the temperature from 15° feeding was diminished, and below 12° almost ceased. A temperature of 14° was selected as the most suitable for the cold-water tank and was maintained throughout the experiment.

Unfortunately, in this first experiment, no record was kept of increases in weight or of food consumption. Each month a sample of mussels was examined for fat content; there was little variation, the total ether-soluble matter averaging about 1.35%. Feeding was continued for 6½ months, when the eels were killed and the body fats extracted. In all the tanks there were a few deaths due to initial injury and to fighting, and in two cases (after 3 months in warm sea water and 5 months in warm fresh water) there was a breakdown of aeration which caused the deaths of about 20 eels in each case, the remainder in each tank recovering. These latter dead eels were also extracted and their body fats examined.

As stated, the eels were originally all about 30–35 cm. in length. It is necessary to have a uniform length to get roughly uniform fat content. The fat content of the eel increases almost linearly with length, at any rate between certain limits (Fig. 1). Thus, on the average, these eels would start with about 9% of fat.

At the end of the experiment some individuals appeared to have grown appreciably, whilst others had not. It is known that the so-called "male" eels do not grow so fast as the female eels, and possibly the differences observed were due to mixed "male" and female eels being used. There was a greater preponderance of larger eels in tank 3 at the end of the experiment than in the other tanks.

The fat contents of the eels are given in Table I and the composition of the fats in Table II. A control sample of eels of the same length obtained from the same source (estuarine waters of the River Dee), is included in both tables.

Fig. 1. Fat content of eels.

Table I. *Fat contents of eels*

Batch no.	Description	Period fed months	Fat content %	Iodine value
1	Control	—	9.3	118.5
2	Fresh water 14°	6½	18.0	119.1
3	„ 23°	5	8.2	126.4
4	„ 23°	6½	10.2	121.5
5	Sea water 23°	3	8.9	118.5
6	„ 23°	6½	9.4	116.6

Table II. *Composition of fatty acids of eel fats (wt. %)*

Batch no.	Saturated			Unsaturated				
	C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂
1	4.3	16.8	2.5	0.1	8.8	39.4	20.8	7.3
					(-2.2 H)	(-2.5 H)	(-5.6 H)	(-10.2 H)
2	3.3	20.5	2.7	Nil	11.6	39.4	16.1	6.4
					(-2.4 H)	(-2.7 H)	(-6.3 H)	(-10.0 H)
3	3.6	17.9	2.8	Trace	6.8	39.2	22.4	7.3
					(-2.3 H)	(-2.6 H)	(-5.8 H)	(-8.0 H)
4	4.1	19.4	2.2	Nil	5.7	37.5	20.6	10.5
					(-2.1 H)	(-2.8 H)	(-5.5 H)	(-9.2 H)
5	3.1	19.8	4.8	0.7	5.8	42.6	15.7	7.5
					(-2.4 H)	(-2.7 H)	(-5.5 H)	(-8.0 H)
6	3.7	17.4	3.9	Nil	9.5	39.6	16.8	9.1
					(-2.1 H)	(-2.4 H)	(-5.2 H)	(-8.6 H)

A large quantity of mussels was extracted, and the ether-extract (123 g.) separated into acetone-soluble fat (73 g.) and acetone-insoluble crude phosphatides (50 g.). The mixture of fatty acids recovered from each fraction was analysed. These mussels were obtained in December 1936. In October 1937 (in connexion with a second experiment) another, larger batch of mussels was extracted, giving 324 g. acetone-soluble fat and 152 g. phosphatides. The original analysis on the fat fraction was satisfactory, but the analysis of phosphatides

was deemed unreliable, so another analysis was carried out on the second batch of phosphatides. In each case appreciable amounts of organic acids of non-fatty nature were encountered, also about 20 % of highly unsaturated acids of high molecular weight (about C_{28} ?). Both phosphatide acid analyses are only approximate in view of the considerable experimental difficulties. These unusual acids did not occur in the acetone-soluble fat. The figures for the mussel lipoids are given in Table III (with non-fatty acids and C_{28} (?) acids excluded).

Table III. *Fatty acids of mussel lipoids (wt. %)*

Material	Saturated				Unsaturated				
	C_{14}	C_{16}	C_{18}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Fat	1.9	16.7	1.7	0.3	10.6	21.5	29.9	13.9	3.5
					(-2.5 H)	(-4.1 H)	(-7.3 H)	(-9.3 H)	(-? H)
Phosphatide (1)*	Nil	28.5	14.4	Nil	3.2	10.1	29.9	13.2	?
					(-3.5 H)	(-4.6 H)	(-6.4 H)	(-8.6 H)	—
Phosphatide (2)	Nil	27.3	6.2	Nil	5.0	16.4	32.2	12.9	?
					(-3.2 H)	(-4.0 H)	(-6.0 H)	(-8.5 H)	—

* Plus 0.7 % arachidic acid.

This first experiment was considered unsatisfactory in several respects—in particular, the lack of records of growth and food consumption, and the use of mixed “male” and female eels with different growth rates. A second experiment was undertaken with the conditions as follows.

(1) The tanks were filled with (a) sea water at 14°, (b) fresh water at 14°, (c) fresh water at 23°.

(2) Each tank was stocked with 25 eels 50–55 cm. in length. This is above the supposed maximum size for “male” eels.

(3) The combined weight of eels in each tank was taken at the start and finish of the experiment.

(4) The diet in the fresh-water tanks consisted of herring. A large supply of herring in uniform condition was obtained at the start of the experiment. This was kept in cold storage and the required amount withdrawn daily. It was fed as pieces of flesh (with skin) about 2 cm. square, containing 20.7 % fat. The diet in the sea-water tank consisted of mussels.

(5) The food was fed *ad lib.*, and the daily consumption was measured.

(6) Any eels which died during the course of the experiment (which ran for 6 months) were weighed and the weight was subtracted from the initial weight. It was assumed that they had eaten nothing since the commencement of the experiment, which was in most cases probably true, owing to initial injuries.

The particulars of the eels are given in Table IV and the composition of their fat in Table V. A control sample of eels of 50–55 cm. length, obtained from the estuary of the Dee, was also examined. The composition of the fat of the herring used is included in Table V. The samples are numbered as follows: 1, control; 2, sea water at 14°; 3, fresh water at 14°; 4, fresh water at 23°. The initial weights are the corrected ones, allowing for 10 deaths in sample 2, 7 deaths in sample 3, and 8 deaths in sample 4.

Table IV. *Particulars of eels in Exp. 2*

Sample	Initial wt. g.	Final wt. g.	Total food eaten g.	Final fat content %	Iodine value
1	—	—	—	24.1	119.0
2	3038	2574	10,459	16.6	114.1
3	2732	3159	4,140	20.6	140.4
4	2829	3556	5,739	23.9	137.6

Table V. *Fatty acid compositions of eel fats in Exp. 2 (wt. %)*

Sample	Saturated			Unsaturated				
	C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂
1	4.3	17.8	1.7	Trace	9.2 (-2.2 H)	38.4 (-2.7 H)	20.1 (-6.0 H)	8.5 (-9.3 H)
2	4.8	17.6	2.6	—	9.4 (-2.2 H)	41.6 (-2.4 H)	16.4 (-5.8 H)	7.6 (-9.1 H)
3	6.4	17.1	1.3	—	6.9 (-2.4 H)	31.9 (-3.0 H)	22.2 (-5.4 H)	14.2 (-7.5 H)
4	6.0	16.5	1.1	0.6	8.3 (-2.2 H)	33.9 (-2.8 H)	22.6 (-5.5 H)	11.0 (-7.0 H)
Herring	8.3	12.1	0.3	0.5	6.4 (-3.4 H)	21.0 (-4.5 H)	28.3 (-5.5 H)	23.1 (-4.6 H)

The eels in the cold sea water were fed on mussels to obtain data on increase (or loss) in weight on a diet so low in fat. In Exp. 1 there was reason to suspect considerable deposition of fat in the cold fresh water, judging by the unusually high fat content (18 %) of the eels. This was the more remarkable since the eels in cold water eat less of any given food than those in warm water. Actually, there was a loss in weight (as well as a reduction in fat content) on a similar diet in cold sea water (Table IV).

An attempt was made to keep eels in sea water on a diet of herring in a smaller tank than the three described. The water was not circulated, but was well aerated and changed daily. For some reason the eels refused to eat anything but particularly tempting food, such as live earthworms. After 5 months' total starvation the attempt was abandoned.

DISCUSSION

It will, perhaps, be best if the influence of the various factors, diet, temperature and salinity, are discussed under separate headings, although some overlapping is inevitable.

Diet. The diets fed, mussels and herring, probably represent about the extremes of non-fatty and fatty food likely to be eaten by an eel in its natural environment. The mussels contained on an average 1.35 % of ether-soluble matter. Allowing for the 15 % of unsaponifiable matter present in this and for the lower proportion of fatty acids in phosphatides than in true fats, the mussels contain about 1.1 % of fat. Not many aquatic organisms will have appreciably less fat than this, and not many will have more than the 20.7 % of fat present in the herring.

Both the dietary fats differ considerably in composition from eel's fat. In the mussel, considerable proportions of phosphatide are present. The acids of the neutral fat and of the phosphatides differ mainly in the presence of more palmitic and stearic acids and less C₁₆ and C₁₈ unsaturated acids in the phosphatides. If total groups are considered (all C₁₆ acids, for instance, being considered together), there is no marked difference between the fatty acid compositions of the three materials included in Table III. Indeed, one could imagine that the glycerides and phosphatides of the mussel had been formed from almost the same mixture of acids, with subsequent selective hydrogenation of the phosphatide mixture. The main differences between the mussel's fatty acids and those of the eel (no. 1 of Table II and also of Table V are very similar) are in respect of the presence of considerably less C₁₈ acids, less C₁₄ and more C₂₀ and

C₂₂ acids in the mussel. The most marked differences between herring's fat and eel's fat are in respect of the presence of much more C₂₂ acids and much less C₁₈ acids in the former. All the other groups, however, are appreciably different in the two fats, and there are more C₁₄ and C₂₀ and less C₁₆ (especially total C₁₆) acids in the herring's fat.

The depot fat of the eels should have been appreciably modified if there had been any considerable incorporation of the ingested fat. From Table II, and also from comparison of samples 1 and 2 in Table V, it can be seen that there was no tendency for the fat of eels on a diet of mussels to be modified in the direction of mussel's fat. This is in accordance with evidence already quoted, that dietary fats differing from the fat of the experimental animal in the relative proportions of the various acids must be fed above a certain level before modification takes place.

On the diet of herring there has been appreciable modification of the eel's fat in the direction of herring's fat (Table V, samples 1, 3 and 4). In fact, the final compositions attained in samples 3 and 4 correspond well with those of definite mixtures of eel's fat and herring's fat. In Table VI are given the calculated compositions of mixtures of 62% eel's fat with 38% herring's fat and 78% eel's fat with 22% herring's fat, expressed as total groups, compared with the actual fats of samples 3 and 4 (Table V) respectively. The analytical method used is not sufficiently accurate, of course, to differentiate between, say, a 60/40 mixture and a 62/38 mixture. Furthermore, the original eel's fat and the ingested herring's fat were probably not absolutely uniform in composition.

Table VI. *Mixtures of eel and herring fats*

Material	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂
62/38 mixture	6.0 (+)	23.8	33.0	23.2	14.0
Sample 3	6.4	24.0	33.2	22.2	14.2
78/22 mixture	5.3	25.1	36.0	21.9	11.7
Sample 4	6.6	24.8	35.0	22.6	11.0

It is evident from Table VI that when the ingested fat does find its way into the fat depots of the eel, it does so with the relative proportions of its various acids practically unaltered. Schoenheimer & Rittenberg's [1935] experiments suggest a deposition of most of the ingested fat in the depots before use, and other experiments [Rittenberg & Schoenheimer, 1937] suggest a continual turnover between depot fat and ingested fat. Whilst this may be true for normal dietary fats, it cannot be unconditionally so for dietary fats differing appreciably from the depot fat of the animal. The results with the eel demonstrate this. On the theory of continual turnover it is possible to calculate the final composition of the eel's fat by assuming that all the ingested fat is deposited in the depots and then a certain amount of the mixed fat is withdrawn.

Errors in the calculation may be caused by the assumption that all the eels in Exp. 2 started with about 24% of fat. Fig. 1 was compiled from results obtained on batches of about 20 eels of each size. As the experimental batches consisted of a fair number of eels in each case, there is a good chance of individual variations being largely compensated. Moreover, it is unlikely that the average of all three batches varied much from 24%. For the sake of the following calculations a uniform initial fat content of 24% in Exp. 2 will be assumed.

From Table IV we can calculate the daily intake of fat and the daily retention. It will not cause serious error to assume a constant daily intake of 1/180th of the total amount eaten. On this basis sample 2 consumed 0.64 g. of mussel's

fat daily, and lost 301.4 g. of their own initial fat, or a total daily loss of 2.31 g. If this 0.64 g. was deposited unchanged in the depots and 2.31 g. of the mixed fat was withdrawn, the final composition after 180 days should be 81 % eel's fat, and 19 % mussel's fat. Such a mixture does not differ from eel's fat sufficiently to be detectable by analysis. Actually, the fat of sample 2 differs slightly from that of sample 1 in the reverse direction from that produced by admixture of mussel's fat with eel's fat. This is presumably due to variability in eel's fat itself and to experimental error. The apparent failure of a diet low in fat to affect the composition of the depot fat, may, therefore, in this instance at least, be due to the limitations of the analytical procedure.

Sample 3 ingested 4.76 g. of herring's fat daily and the daily loss of fat was 4.78 g. By the above hypothesis this process would finally give a mixture of 28 % eel's fat and 72 % herring's fat, contrasted with the 62 % eel's fat: 38 % herring's fat mixture actually found. Again, in sample 4 there was a daily ingestion of 6.60 g. of herring's fat and a daily loss of 5.65 g., which should ultimately give 24 % eel's fat and 76 % herring's fat, contrasted with the 78 % eel's fat: 22 % herring's fat mixture found. Such differences could readily be detected by analysis.

The differences are so large that it seems improbable that they can be attributed to error in the assumption of an initial fat content of 24 %. An initial fat content of 20 %, for instance, gives final compositions of the same order as with the 24 % figure. The absorption of the mussel's and herring's fats by the eels should be almost complete. McCay & Tunison [1934], in experiments on trout, showed good absorption of various fats fed even at 25 % of the diet. The conclusion of the writer is that there cannot have been a quantitative turnover between ingested fat and depot fat.

The hypothesis of Schoenheimer & Rittenberg [1935] may be open to question. If admixture with the fat already in the depots is thorough before withdrawal of mixed fat for combustion, then in the case of mice fed at the 1 % level for 4 days one would expect to find about 95 % of the dietary fat in the depots instead of the 47 % actually found. It is possible, of course, that the incorporation of ingested fat in the depots is not uniform, and that the recently deposited fat is more readily withdrawn than the rest of the depot fat. Either of these conditions (i.e. partial deposition only or uneven admixture and withdrawal), or perhaps both together, could account for the observed results on eels not being fully quantitative.

An interesting point is the high fat content of eels in batch 2, Table I. Although no records of weight were taken, there was visible evidence of growth, and this on a diet of mussels. The reason is obscure, but it may be noted that the final fat showed no evidence of having been modified by the diet. The eels feeding on mussels in Exp. 2 failed to maintain weight.

Although the idea of a complete interchange between ingested fat and depot fat is not in harmony with the evidence, neither is the idea that there has been no interchange, and that the only herring's fat deposited is that represented by the total gain in fat on the diet of herring. On this basis the final fat of sample 3 (second experiment) should have consisted of entirely unchanged eel's fat.

Evidently the quantitative effect of the dietary fat on the depot fat is governed by factors which are still obscure. The metabolic rate appears to have something to do with it, since the warm-water fish, in spite of ingesting more herring's fat than did the cold-water fish, did not modify their fat so much.

Temperature. In considering the effects of temperature we should not compare various samples with the control estuarine sample, since the temperature

of the estuary was variable and unknown. In Table II we can compare sample 2 with samples 3 and 4, where the only difference was one of 9° in temperature. There is a slight suggestion that the fat in cold water is more unsaturated (certainly not shown by the iodine value, however, as seen from Table I). C_{16} , C_{20} and C_{22} unsaturated acids are slightly more unsaturated in sample 2, whilst C_{18} acids are about the same. There is evidence of the production of a more saturated fat at higher temperatures, but the effect is certainly not a large one.

In the second experiment we may compare samples 3 and 4, with similar results. C_{16} , C_{18} and C_{22} acids are slightly more unsaturated in the cold water than in the warm water, C_{20} acids being about the same. In this experiment, however, the position is complicated by the different amounts of herring's fat which have been incorporated in the depots. Herring's fat is more unsaturated than eel's fat for C_{16} and C_{18} acids, and less unsaturated for C_{22} acids. C_{20} acids are about the same (if both samples of estuarine eel's fat are considered). The fact that less herring's fat has been incorporated in the fat of the eels in sample 5 than in that of the eels of sample 4 would mean that the results for C_{16} and C_{18} acids could be accounted for on this basis. The results for C_{22} acids could not, however, be so interpreted and so here the evidence supports that of Exp. 1.

Actually, whilst as far as total groups are concerned the herring's fat appears to be deposited with the relative proportions unchanged, the degrees of average unsaturation show distinct changes. In Table VII are given the degrees of average unsaturation of C_{16} , C_{18} and C_{22} unsaturated acid groups.

Table VII. *Degrees of unsaturation of eel fats*

Material	Degrees of unsaturation		
	C_{16}	C_{18}	C_{22}
62% eel fat 38% herring fat }	-2.7 H	-3.3 H	-7.5 H
Sample 3	-2.4 H	-3.0 H	-7.5 H
78% eel fat 22% herring fat }	-2.5 H	-3.2 H	-8.5 H
Sample 4	-2.2 H	-2.8 H	-7.0 H

It can be seen from Table VII that the eels in both warm and cold water have partially hydrogenated either the herring's fat itself or more probably the mixed fat in the depots. In the cold water such hydrogenation has been confined to the C_{16} and C_{18} acids—the ones which the diet would tend to make more unsaturated. However, in the warm water the C_{22} acids are also hydrogenated, and this difference is presumably due to the higher temperature. The extent to which the other acids are hydrogenated is almost the same in the two samples.

Both experiments, therefore, afford evidence of greater unsaturation in fats laid down at lower temperatures, but the effect is certainly not a marked one. It is notable that whilst in both samples 3 and 4 hydrogenation has gone far enough to bring the unsaturation of the C_{16} and C_{18} acids almost to normal (Table V), there is not hydrogenation to such an extent that the iodine value of the entire fat is normal (Table IV). This is a further illustration of the comparative unimportance of the iodine value as a guide to metabolic processes in fats.

The principal effect of a higher temperature in Exp. 2 has been to accelerate the process of modifying ingested fat.

Salinity. From the results of Exp. 1 it is evident that under such conditions salinity has had no effect. Comparing samples 5 and 6 with 3 and 4 (Table II), there is no evidence at all of a tendency towards a marine type of fat in sea water or a more truly fresh-water type in the fresh water. The differences between these types have been illustrated in detail [Lovern, 1937], and consist of higher proportions of C_{16} and especially C_{18} acids, and lower proportions of C_{20} and especially C_{22} acids, in the fats of fresh-water fish. The estuarine eels' fats (controls) correspond fairly well to the fresh-water type, but are not really typical—in particular the C_{16} acids content is on the low side and the C_{20} acids content on the high side. The reason may be that they were obtained from brackish estuarine waters. That such a habitat may lead to a modified fat was suggested by the author [Lovern, 1937]. Shorland & McIntosh [1936] found that a closely related eel from New Zealand had fat resembling that of typical fresh-water fish.

It should perhaps be noted that in these experiments aeration would probably not be as complete as under natural conditions. If the differences in metabolic rate observed by Fox & Simmonds [1932] are due to the greater oxygen content of fresh water compared with sea water (as they suggest), such differences might not obtain in the experimental tanks.

It seems doubtful whether any fat was laid down under the conditions of Exp. 1 (apart from batch 2). It might be argued that the failure of salinity to have any effect was due to this, and that only fat actually deposited whilst the fish are in a certain environment can be affected by that environment. It has been shown [Lovern, 1934] that starving salmon in fresh water do not modify their fat in the direction of the fresh-water type. These eels were not starving, however, and unless the depot fat remained in a static condition with no ebb and flow (and the experiments on feeding herring definitely indicate an ebb and flow), it seems hard to avoid the conclusion that salinity has little if any direct effect.

The experiments with a diet of herring are difficult to interpret. Herring's fat is markedly marine in type, and yet 6 months' feeding on this fat at as high a level as is ever likely to be encountered in nature, did not make the eel's fat truly marine in type. It is notable that the more intensive feeding in the warm water, so far from causing the fat to become still more marine, actually resulted in less change. This suggests that there is in the animal a mechanism which can deal with ingested fat so as to modify it to the normal requirements of its depot fat. This mechanism might be unable to deal with fat above a certain level of ingestion, but its activity might well be increased at higher temperatures.

It is impossible to tell from these experiments how far it would be possible to modify the fat of a fish by an unusual diet if that diet was fed throughout its life. On the other hand, the existence of a mechanism which can modify ingested fat to a certain extent, and the fact that the rate at which this mechanism functions can be altered (e.g. by changes of temperature) is significant. Such a mechanism, with a variable rate, has been adduced for the herring [Lovern, 1938].

SUMMARY

Using eels (*Anguilla vulgaris*) as experimental animals, an attempt has been made to determine the separate effects of diet, temperature and salinity on the composition of the depot fats of fish.

On a diet low in fat (1.1%), the ingested fat had no detectable effect on the depot fat. On a diet high in fat (20.7%), the eel's depot fat was appreciably modified, but the quantitative relationship between the amount ingested and the effect produced was obscure. Such ingested fat as was incorporated in the depot

fat was apparently deposited with the relative proportions of the various acids practically unchanged. Hydrogenation, however, had taken place. The eel possesses a mechanism which can modify ingested fat to a considerable extent, and the action of this mechanism is accelerated by rise of temperature.

In addition to the above property, temperature has a direct effect on the composition of fat. Lower temperatures lead to more unsaturated fat and higher temperatures to increased saturation. This effect is relatively a small one, and in natural conditions cannot be important.

The effect of salinity has not been thoroughly settled, but with a low intake of fat it appears to have no direct significance.

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