# CCXXXIV. FAT METABOLISM IN FISHES.

## I. GENERAL SURVEY OF THE FATTY ACID COMPOSITION OF THE FATS OF A NUMBER OF FISHES, BOTH MARINE AND FRESH-WATER.

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The problems of fat metabolism in fishes appear in several ways to be somewhat different from those met with in the study of fat metabolism in either plants or mammals. The mixtures of fatty acids which go to form the fat of plants or mammals are much simpler than those of fishes, and it is difficult to find a reason for the special compositions of fish fats, with their numerous unsaturated components and high molecular weights. Two factors are probably of importance.

(*a*) Cold-blooded animals in general lay down a more unsaturated type of fat than warm-blooded ones. The effects of temperature on degree of unsaturation, as affecting both plant and animal depôt fats, are well established. This, however, will not explain the presence of fatty acids of such high molecular weight. It also does not account for the fact that the fats of marine mammals are mostly similar in general composition to those of the cold-blooded fishes.

Of the common marine species most are exclusively carnivorous, eating other fish. Thus their own fat composition will be decided largely by the type of fat in their food, since their carbohydrate intake is low, and fat synthesis from this source is not likely to be great. True, since protein can be transformed into carbohydrate, there is a possibility of fat being formed from protein, but the process seems an extravagant and wasteful one, and the writer is unaware of any evidence in support of this hypothesis. Thus it seems evident that if the smallest marine animals, which feed on marine flora, lay down these special fats, they will automatically bring about the storage of closely allied fats in the larger fish. It is therefore of interest to note that the marine flora contains poly-unsaturated fatty acids of high molecular weight similar to those found in the fat of fishes, although in very small amounts [Tsujimoto, 1925].

The number of species of fish whose fat has so far been quantitatively investigated is small, and it is to be noted that no single fresh-water species is included. This is significant in view of the possibly different food supply of most fresh-water fish.

In 1930, Guha *et al.* [1930] published a paper in which was given a list of the quantitative compositions of the fatty acids of all the fish fats examined up to that date. This included only 13 species, and the authors pointed out that it was important that their survey should be greatly extended, in order to substantiate the biological group classifications which they made. This paper was followed by one from the writer [Lovern, 1930], in which further support was given to the previous authors' suggestions as to the peculiar relationships holding in the Elasmobranch family. Since that date the writer has not found opportunity to pursue this particular point further, but it is certainly one which will require a more detailed consideration. The only other quantitative data which the writer has observed are the partially quantitative analysis by Tsujimoto [1932] of a shark-liver oil of low iodine value, and the earlier work of Gill and Tucker [1930] on the composition of porpoise-jaw oil. The existence of such an oil as the last mentioned, of which the characteristic acid is *iso*valeric, illustrates one of the extraordinary complexities of fat metabolism in fishes.

The methods employed in the present work were practically identical with those given in detail in earlier papers [Guha *et al.*, 1930] and will not be described here. Considerations of space also preclude the giving of all the analytical data in detail, and only the final compositions will be included. One slight modification of method deserves mention. In the process of separating into liquid and solid acids by lead salt/alcohol, Banks [1932] found a much more complete separation when 0·5 % of acetic acid was added to the alcohol in the first precipitation. (This is, of course, always added of necessity in the subsequent recrystallisation of the solid lead salts.) The writer has used this new process with entire success.

In Table I are given the particulars of the oils, and in Tables II and III the percentage compositions of the fatty acid mixtures present in the new marine species examined. Table II is expressed in weight percentages, since this is the way in which previously recorded compositions have invariably been given, and Table III is expressed in molar percentages. In Table III is included an average of the previous group classifications, brought to molar percentages. The advantage of considering results from a molecular standpoint,

Table I. *Particulars of marine oils examined.*

| Species | Description of oil |
|---|---|
| Haddock (*Gadus aeglefinus*) | Oil from the liver, the main storage depôt<br>Iodine value 168·5 and unsaponifiable matter 0·7 % |
| Sprat (*Clupea sprattus*) | Oil from the head and body tissues, the main storage depôt<br>Iodine value 150·7 and unsaponifiable matter 0·9 % |
| Angler (Monk) fish (*Lophius piscatorius*) | Oil from the liver, the main storage depôt<br>Iodine value 162·3 and unsaponifiable matter 1·0 % |
| Halibut (*Hippoglossus vulgaris*) | Oil from the liver, one storage depôt<br>Iodine value 120·0 and unsaponifiable matter 6·6 % |

Table II. *Composition of mixed fatty acids (weight percentages) of oils of marine species.*

| | Saturated | | | Unsaturated | | | | |
|---|---|---|---|---|---|---|---|---|
| Species | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{22}$ |
| Haddock | 4·3 | 14·1 | 0·3 | 0·5 | 12·4 | 30·5 (−2·6 H) | 29·3 (−5·9 H) | 8·6 (−7·3 H) |
| Sprat | 6·0 | 18·7 | 0·9 | 0·1 | 16·2 | 29·0 (−2·9 H) | 18·2 (−5·6 H) | 10·9 (−7·1 H) |
| Angler fish | 4·9 | 9·6 | 1·3 | 0·4 | 12·1 | 30·9 (−3·3 H) | 24·9 (−5·9 H) | 15·9 (−8·6 H) |
| Halibut | 3·9 | 15·1 | 0·5 | Nil | 18·7 | 34·4 (−2·0 H) | 13·8 (−5·5 H) | 13·6 (−7·6 H) |

Table III. *Composition of mixed fatty acids (molar percentages) of oils of marine species.*

| | Saturated | | | Unsaturated | | | | |
|---|---|---|---|---|---|---|---|---|
| Species | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{22}$ |
| Haddock | 5·2 | 15·4 | 0·3 | 0·6 | 13·6 | 30·5 (−2·6 H) | 27·1 (−5·9 H) | 7·3 (−7·3 H) |
| Sprat | 7·2 | 20·1 | 0·9 | 0·1 | 17·6 | 28·5 (−2·9 H) | 16·5 (−5·6 H) | 9·1 (−7·1 H) |
| Angler fish | 6·0 | 10·6 | 1·3 | 0·5 | 13·5 | 31·2 (−3·3 H) | 23·2 (−5·9 H) | 13·7 (−8·6 H) |
| Halibut | 4·7 | 16·3 | 0·5 | Nil | 20·4 | 34·0 (−2·0 H) | 12·6 (−5·5 H) | 11·5 (−7·6 H) |
| Gadidae* | 6·5 | 12·0 | Trace | Trace | 15·5 | 29·0 | 25·5 | 11·0 |
| Clupidae* | 7 | 14·0 | 1·5 | Nil | 15·5 | 27·0 | 21·0 | 13·0 |
| Balaenidae* | 7·5 | 11·5 | 3·0 | 1·0 | 18·0 | 38·5 | Variable | 10·5 |

* Summarised from work of Guha *et al.* [1930]. Figures for Elasmobranch group, and sperm whale oils not included.

rather than one of mass has been pointed out by Hilditch and Sleightholme [1930].

The average unsaturation of the various groups has been expressed in terms of the number of hydrogen atoms required for saturation, as in previous papers. These values are in agreement with those previously recorded [Guha *et al.*, 1930] for the Gadidae and Clupidae oils. It is to be noted that each oil was a representative sample from a large number of fish, but even so, seasonal and other changes will account for minor fluctuations. In general, it will be seen that the oil from haddock-liver falls quite well into the Gadidae group, and that of the sprat into the Clupidae. These two groups are similar in composition. Angler fish-liver oil is similar to both. Halibut, however, is peculiar in that linoleic acid appears to be absent, and the proportions of acids of the $C_{20}$ and $C_{22}$ groups are nearly the same. Normally the $C_{20}$ group greatly predominates. This seeming lack of linoleic acid will be referred to again. Conjointly with the reduced amount of acids of the $C_{20}$ group it will account for the low iodine value of halibut-liver oil.

Up to the present five oils from fresh-water fish (4 species) have been examined in detail, and in Tables IV, V and VI are given the particulars and the compositions.

Table IV. *Particulars of oils from fresh-water fish.*

| Species | Description of oil |
|---|---|
| Carp (*Cyprinus carpio*) | Oil from head and body tissues, the main fat depôt<br>Iodine value of oil = 122·9 and unsaponifiable matter = 3·5 % |
| Pike (*Esox lucius*) | (*a*) Oil from head and body tissues, one of two main fat stores<br>Iodine value of oil = 145·9 and unsaponifiable matter = 4·0 %<br>(*b*) Oil from the mesentery, the other main fat depôt<br>Iodine value of oil = 134·2 and unsaponifiable matter = 0·1 % |
| Pollan (*Coregonus pollan*) | Oil from head and body tissues, the main fat depôt<br>Iodine value of oil = 136·8 and unsaponifiable matter = 1·5 % |
| Perch (*Perca fluviatilis*) | Oil from head and body tissues, the main fat depôt<br>Iodine value of oil = 131·7 and unsaponifiable matter = 6·0 % |

Table V. *Composition of mixed fatty acids (weight percentages) of oils of fresh-water species.*

| | Saturated | | | Unsaturated | | | | |
|---|---|---|---|---|---|---|---|---|
| Species | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{22}$ |
| Carp | 3·7 | 14·6 | 1·9 | 1·0 | 17·8 | 45·8 (−3·2 H) | 15·2 (−6·9 H) | Nil |
| Pike (body) | 4·7 | 13·2 | 0·5 | 0·8 | 20·8 | 38·4 (−2·8 H) | 15·3 (−7·5 H) | 6·3 (−7·5 H) |
| Pike (mesentery) | 2·9 | 15·0 | Trace | 0·5 | 20·2 | 42·4 (−3·4 H) | 15·1 (−6·7 H) | 3·9 (−8·0 H) |
| Pollan | 2·9 | 14·3 | 1·9 | 1·5 | 19·8 | 40·0 (−3·2 H) | 13·5 (−7·4 H) | 6·1 (−9·1 H) |
| Perch | 3·5 | 12·5 | 2·0 | 1·1 | 19·4 | 40·5 (−3·2 H) | 13·8 (−6·8 H) | 7·1 (−9·2 H) |

Table VI. *Composition of mixed fatty acids (molar percentages) of oils of fresh-water species.*

| | Saturated | | | Unsaturated | | | | |
|---|---|---|---|---|---|---|---|---|
| Species | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{22}$ |
| Carp | 4·4 | 15·5 | 1·8 | 1·2 | 19·0 | 44·5 (−3·2 H) | 13·6 (−6·9 H) | Nil |
| Pike (body) | 5·6 | 14·1 | 0·5 | 1·0 | 22·4 | 37·4 (−3·0 H) | 13·8 (−7·5 H) | 5·2 (−7·5 H) |
| Pike (mesentery) | 3·4 | 16·0 | Trace | 0·6 | 21·7 | 41·5 (−3·4 H) | 13·6 (−6·7 H) | 3·2 (−8·0 H) |
| Pollan | 3·4 | 15·5 | 1·8 | 1·8 | 21·2 | 39·0 (−3·2 H) | 12·2 (−7·4 H) | 5·1 (−9·1 H) |
| Perch | 4·2 | 13·4 | 1·9 | 1·3 | 20·9 | 39·8 (−3·2 H) | 12·5 (−6·8 H) | 6·0 (−9·2 H) |

Comparing these data with those for the marine oils (Table III), we find:

(*a*) The oleic-linoleic acid group percentage consistently higher in fresh-water fish fats.

(*b*) The $C_{20}$ and $C_{22}$ groups present in considerably smaller amounts in fresh-water fish fats.

(*c*) Palmitoleic acid is a more important component of all the fresh-water fats than of the marine ones, with the exception of halibut-liver oil, which is unique.

(*d*) Otherwise, the fresh-water and marine oils are similar, in that in each class the average unsaturation of the various groups is of the same order, and the general proportions of the fatty acid mixture are the same, *viz.* small quantities of myristic acid, with traces only of myristoleic acid; considerably greater quantities of palmitic and palmitoleic acids (in comparable amounts); traces only of stearic acid, with oleic and linoleic acids as one of the main components of the oils; the $C_{20}$ group, having no saturated member, also a major constituent, and the $C_{22}$ group, still more unsaturated, in smaller amount.

Banks and Hilditch [1932], working on the body fats of the pig, have shown that there is a balance between stearic and oleic acids, tending to keep the total amount of $C_{18}$ acid approximately constant, however much either component varies. Stearic acid is not a major component of fish oils, but palmitic is, and it is of interest to compare the total group percentages (molar) for all the oils described.

Table VII. *Total groups of acids. Molar percentages.*

| Species | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{22}$ |
|---|---|---|---|---|---|
| Marine: | | | | | |
| Haddock | 5·8 | 29·0 | 30·8 | 27·1 | 7·3 |
| Sprat | 7·3 | 37·7 | 29·4 | 16·5 | 9·1 |
| Angler fish | 6·5 | 24·1 | 32·5 | 23·2 | 13·7 |
| Halibut | 4·7 | 36·7 | 34·5 | 12·6 | 11·5 |
| Gadidae (average) | 6·5 | 27·5 | 29·0 | 25·5 | 11·0 |
| Clupidae (average) | 6·0 | 27·2 | 28·7 | 22·5 | 15·5 |
| Fresh-water: | | | | | |
| Carp | 5·6 | 34·5 | 46·3 | 13·6 | Nil |
| Pike (body) | 6·6 | 36·5 | 37·9 | 13·8 | 5·2 |
| Pike (mesentery) | 4·0 | 37·7 | 41·5 | 13·6 | 3·2 |
| Pollan | 5·2 | 36·7 | 40·8 | 12·2 | 5·1 |
| Perch | 5·5 | 34·5 | 41·7 | 12·5 | 6·0 |

It will be noticed that there is a tendency towards an approximate constancy of the amount of $C_{16}$ acids in the fresh-water fish, but no constancy in the marine species. This may, however, be fortuitous with so few samples. It may be significant that the two marine fats with total $C_{16}$ acids of 37 % (sprat and halibut) are also comparable with the fresh-water fats in having a low $C_{20}$ content.

In the paper of Guha *et al.* [1930] it is stated that the poly-unsaturated acids of high molecular weight characteristic of fish oils are evidently marine in origin. This cannot now be accepted unreservedly—they appear to be characteristic of aquatic life (including marine algae, *etc.*) of all kinds, including fresh-water. The diet of fresh-water fish is perhaps different from that of marine species. Certainly many are purely carnivorous, but in many cases they eat such species as the young of small birds (the particular pike examined, for instance, all had small wild-ducklings in their stomachs), frogs, and even mice, *etc.* Others are herbivorous. It has not been shown whether the small crustacea, worms, *etc.*, and the flora of fresh-water contain these typical fish oil acids. It is hoped later to investigate this point.

The high content of oleic and linoleic acids in the fats from the fresh-water fish is paralleled in the marine mammalia (Balaenidae), but the parallelism does not extend to the other groups of acids. However, in all land mammals, the oleic-linoleic acid group is the only unsaturated one present, and hence we may say that the fats from fresh-water fish, as well as those from marine mammals, are of a type intermediate between the fats from marine and terrestrial animals. Differences of food might account for this, if the natural tendency of all fish was to lay down fats of the marine type. It is hoped later to conduct controlled feeding experiments on both fresh-water and marine species to decide this point, but the technical difficulties are considerable. Two other factors must be borne in mind. The average temperature of fresh-water ponds and rivers, especially shallow ones, is likely to be higher than that at the bottom of the sea. Also, the salinity of sea-water may have some effect, although the writer does not consider this probable. It can fairly easily be determined with species which can be kept in both fresh and salt water, *e.g.* eels. It is well not to underestimate the effects of environmental and seasonal differences, as contrasted with the effects of food differences, since the work of Hilditch and Sleightholme [1930] has shown that, in the case of butter fats at all events, the former may far outweigh the latter.

The apparent lack of linoleic acid in the case of halibut-liver oil requires further notice. It is now almost certain that mammals, for example, cannot synthesise linoleic or linolenic acids from carbohydrate, and that when deprived of them in their diet they suffer in health. Administration of either acid will alleviate the trouble [Evans and Lepkovsky, 1932; Burr, Burr and Miller, 1932]. Earlier work on different lines by Ellis and Harkins [1925] on the composition of pig fats lends decided support to this view, that all the linoleic acid present has come directly from ingested linoleic acid. The results of Hilditch and his collaborators [Banks and Hilditch, 1932; Bhattacharya and Hilditch, 1931] on pig fats are also in accordance with this theory. There is no evidence as to whether or not linoleic acid is essential to the well-being of fishes, but it is significant to note that the highly unsaturated arachidonic acid did not alleviate the symptoms in the experiments of Burr, Burr and Miller [1932]. Either linoleic acid is not essential to the halibut (it must be remembered that the diet of the halibut will include linoleic acid), and is hence readily destroyed by oxidation, or else it was present, but in traces too small for detection by the analytical methods employed. There is a third possibility. The liver is not the only fat store in the halibut, and there are quite considerable amounts of fat deposited just beneath the skin. It would be necessary to analyse fat from every single depôt in the fish before it could be said that linoleic acid was definitely absent.

Nevertheless, the composition of halibut-liver oil is unusual in this respect, and also in its low content of acids of the $C_{20}$ series. The writer is frequently examining samples of halibut-liver oil in connection with another piece of work, and has found that the iodine value is invariably low, and of the order of 120, as with the sample analysed. Thus the possibility of it being a "freak" sample is rather remote.

One other point is worthy of mention. It was indicated in the earlier work of Guha *et al.* [1930] that the widely varying extents to which the highly unsaturated esters tended to polymerise on heating suggested that the structures of the acids were not necessarily similar in the different oils. The writer has observed the same marked peculiarities in the present work, and also a possibly associated fact, that with some oils the highly unsaturated esters which distil over have a deep golden-yellow colour, whilst with others the distillates are almost colourless. This is quite irrespective of the iodine value of the fractions, or of the colours of the original oils, and is not due to traces of pigment. This point seems worthy of further investigation, and is being followed up.

## Summary.

A start has been made on a series of investigations into the peculiar problems of fat metabolism in fishes. The work of Guha *et al.* [1930] has been extended as a preliminary to more detailed work, and the present paper includes data for a number of oils, including five from fresh-water fish.

Significant differences in fatty acid composition are apparent between the oils from fresh-water fish on the one hand, and from marine species on the other. The most noticeable are the increased proportions of oleic and linoleic acids, and also of palmitoleic acid, in the fresh-water fish oils, and the reduced amounts of acids of the $C_{20}$ and $C_{22}$ series. Possible reasons for this are mentioned.

The composition of a sample of halibut-liver oil, representative of many fish, has been shown to be unique in respect of an apparent entire lack of linoleic acid, and of the low ratio of $C_{20}$ to $C_{22}$ acids. The significance of this absence of linoleic acid is discussed.

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