LXII. FAT METABOLISM IN FISHES.

IX. THE FATS OF SOME AQUATIC PLANTS.

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It has been shown [Lovern, 1935, 1] that fats from several plankton crustacea exhibit the same type differences between marine and freshwater species as are shown by fish fats, namely more C_{16} and C_{18} and less C_{20} and C_{22} unsaturated acids in the freshwater species. Indeed, so far as the animal chain is concerned, from the vegetable-feeding plankton upwards, the evidence is in harmony with the idea that ingested fat may be deposited largely unchanged in most cases. It is of interest to know whether the plankton crustacea may also deposit ingested fat unchanged, or whether they are forced to modify it. There is also the question of whether the type difference between marine and freshwater species is confined to the animal kingdom or extends to aquatic plant life.

In the present paper the results of the detailed analyses of the fats from several species of algae, both marine and freshwater, are described. In addition the fats from a higher aquatic plant and from a marine diatom have been examined. So far as the author is aware the only data so far available regarding such fats are qualitative or at most semiquantitative. The content of fatty matter in marine algae has been shown to depend mainly on the depth of immersion [Russell-Wells, 1932; Haas and Hill, 1933]. Tsujimoto [1925] showed by bromination that algal fats contained small quantities of highly unsaturated acids. Takahashi *et al.* [1933] identified *n*-hexanoic, *n*-octanoic, *n*-decanoic, linoleic, oleic, and myristic acids in certain algal fats. Collin *et al.* [1934] found that fatty acids from mixed phytoplankton gave only a small yield of ether-insoluble bromides.

EXPERIMENTAL.

Large quantities of the marine algae *Fucus vesiculosus*, *Laminaria digitata*, *Rhodomenia palmata* and a small quantity of mixed green species were collected near Aberdeen. Green marine algae are not easy to obtain in quantity, and the mixture was needed to obtain sufficient fat for analysis. Supplies of the freshwater algae *Nitella opaca* and *Oedogonium* sp. were supplied through the kindness of Dr R. L. A. Beauchamp of the Freshwater Biological Association of the British Empire. With the assistance of the Great Yarmouth Port and Haven Commissioners a large supply of the freshwater alga *Cladophora sauteri* was obtained from Hickling Broad. The higher aquatic plant *Anacharis alsinastrum* (Canadian pondweed) was obtained in quantity from the Loch of Pitfour, Aberdeenshire, by the kind permission of Mr F. Martin of Mintlaw, Aberdeenshire. The marine diatom *Nitzschia closterium* was grown in pure culture until about 13 g. of fat were available.

The algae and the pondweed (entire plant) were all extracted with ether after air-drying and powdering. The *F. vesiculosus* was extracted at Manchester by Prof. I. M. Heilbron in the course of his work on algal constituents and the fatty acids were forwarded to Aberdeen. In this case two extractions were made,

first with alcohol and then with ether, the fatty acids from each of these extracts being examined separately. All the other dried materials were extracted with ether only and in every case except the pondweed the extract was freed from phosphatides with acetone. The proportions of phosphatides were always very low and the pondweed results can safely be taken as those of the glyceride fraction only. The diatoms were filtered out and the whole mass, including paper, was extracted first with alcohol and then, after mincing, with ether. The extracts were combined and this fat was also freed from phosphatides.

The samples were examined by substantially the same process as that used hitherto for fish fats, but the presence of large quantities of chlorophyll (which cannot be altogether removed during saponification, removal of unsaponifiable matter and acid recovery) necessitated purification of the fatty acids with light petroleum. Small quantities of chlorophyllins which remained accumulated in the residues during distillation and were removed by again extracting the residues with light petroleum. The possible presence of lower fatty acids such as *n*-decanoic acid also involved extra precautions. It was further found that the pondweed fat was contaminated with appreciable quantities of wax acids (both saturated and unsaturated) similar to those reported in certain grass fats [Lovern, 1935, 2]. All but one of the algae appeared to be free from waxes, but *C. sauteri* contained traces. The fat contents of the various materials were not accurately determined, but it was evident that the depth effect previously mentioned was correct.

The results of the fatty acid analyses are given in Table I. The wax acids have been eliminated from the two cases where they were present, and in every case the composition recorded is that of the glyceride fatty acids. These plant fats are

Table I. *Composition of mixed fatty acids (wts. %).*

Class	Species	Saturated				Unsaturated				
		C _{<14}	C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂
Chlorophyceae	<i>N. opaca</i> *	—	6	18	3	3 (> -2.0H)	34 (-2.5H)	23 (-4.5H)	13 (-5.8H)	—
	<i>Oedogonium</i> sp.*	—	2	20	1	—	32 (-3.1H)	35 (-4.6H)	9 (-?H)	1 (-?H)
	<i>C. sauteri</i> *	Trace	12	10	2	Trace	19 (-4.7H)	49 (-3.8H)	8 (-7.1H)	—
	Mixed†	—	4	10	2	3 (> -2.0H)	39 (-3.4H)	30 (-5.1H)	8 (-6.5H)	4 (-?H)
Phaeophyceae	<i>F. vesiculosus</i> (1)†	Trace	8	9	1	Trace	6 (-2.0H)	57 (-3.2H)	16 (-7.3H)	3 (-?H)
	<i>F. vesiculosus</i> (2)†	Trace	9	7	2	1	5 (-2.0H)	63 (-3.0H)	13 (-7.3H)	—
	<i>L. digitata</i> †	—	6	14	1	2	11 (-2.0H)	42 (-4.2H)	24 (-8.1H)	—
Rhodophyceae	<i>R. palmata</i> †	1	4	19	1	Trace	6 (-2.9H)	20 (-4.5H)	36 (-9.2H)	13 (-?H)
Higher plant	<i>A. alsinastrum</i> *	1	1	15	5	2	25 (-3.0H)	39 (-4.9H)	12 (-6.0H)	—
Diatom	<i>N. closterium</i> †	—	8	17	2	1	36 (-3.4H)	20 (-5.3H)	16 (-7.0H)	—

* Freshwater species.

† Marine species.

more difficult to deal with and the experimental error is likely to be higher than with fish fats. In addition several fats had to be examined on a small scale and so the results are only expressed to the nearest unit. The degrees of average unsaturation are given in terms of lack of hydrogen.

The nature of the acids of less than 14 carbon atoms was not determined in most cases, but in *R. palmata* both *n*-decanoic and *n*-octanoic acids and also smaller traces of an unsaturated acid of less than 14 carbon atoms were present. *F. vesiculosus* (1) and (2) are from the alcohol and ether extracts respectively.

DISCUSSION.

From Table I it can be seen that for these plant fats the distinction between freshwater and marine species does not hold. In the case of the algae the properties seem to follow the colour grouping. Of the green algae three were freshwater species and one marine. This mixed marine specimen was really from brackish water rather than pure seawater, since green algae grow more prolifically near the mouths of rivers than in pure seawater, and this sample was collected in such brackish areas. However, if salinity has any effect on the fat it should have shown in this sample and it is obvious that actually this fat is of the same type as those of the other Chlorophyceae.

The saturated acid percentages show great irregularities throughout, but for the unsaturated acids some interesting correlations occur. For the green algae (Chlorophyceae) the predominating unsaturated acids are those of 16 and 18 carbon atoms, with little C_{20} and little or no C_{22} acids. The degrees of average unsaturation of the C_{16} and C_{18} acids are unusually high. In the brown algae (Phaeophyceae), C_{16} unsaturated acids have not the same importance, C_{18} acids are outstanding and C_{20} acids are present in somewhat greater proportions than for most green algae. The C_{16} acid is monoethylenic only, and the C_{18} acids are not on the whole of an unusually high degree of unsaturation. For the one red alga (Rhodophyceae) examined C_{20} acids are the major constituent and appreciable quantities of C_{22} acids are present. C_{16} unsaturated acids, whilst not present in large amounts, are again of a relatively high degree of average unsaturation.

It is perhaps not unexpected that plant fats will fall into classes according to botanical similarities rather than according to habitat, since this is so markedly the case for seed fats. It is interesting to note that Heilbron *et al.* [1935] found that the components of the unsaponifiable matter from algae were decided by whether the alga was a green, red or brown species.

Turning to the higher plant *Anacharis alsinastrum* we find a fatty acid mixture very similar to that of green algae. The whole question of the composition of leaf, stalk *etc.* fats requires examination, especially in view of the author's results on two land grass fats [Lovern, 1935, 2] where a somewhat similar type of fat was found.

The marine diatom *Nitzschia closterium* has a fatty acid mixture also closely resembling that of a green alga, although this diatom is brown in colour.

Taking these fats as a whole it may be said that the fats of all the green algae, the pondweed and the diatom are of a type very similar in many respects to freshwater animal fats. The brown algal fats are really of a class by themselves, but more like a freshwater than a marine animal fat. The red algal fat is the only one approximating in composition to a marine animal fat.

It has already been mentioned that throughout the animal chain to the copepods and other plankton crustacea, the same general types of fat are found for freshwater and marine organisms respectively. These plankton crustacea do not live on the large attached algae, but on diatoms and the smaller floating algae. If *N. closterium* is typical of most marine diatoms it is evident that such copepods as *Calanus finmarchicus*, for example, must considerably modify ingested fat. *C. finmarchicus* contains high percentages of C_{20} and C_{22} acids [Lovern,

1935, 1]. The work of Collin *et al.* [1934] suggests that *N. closterium* is typical of other marine diatoms since a mixture of forms gave fatty acids of which only 2.1 % gave bromides insoluble in ether. C₂₀ and C₂₂ highly unsaturated acids could not have been present in large amounts, especially as some of the C₁₈ acids (if similar to those of *N. closterium*) would have contributed to the ether-insoluble bromides. If the small floating algae resemble the larger attached forms, the marine zooplankton cannot obtain much of its C₂₂ acids from them either.

Turning to the freshwater organisms it is regrettable that attempts made to grow a freshwater diatom in pure culture were unsuccessful, and so no analysis comparable with that of *N. closterium* fat is available. However, it has been shown that for plants the distinction between freshwater and marine forms does not hold, but that botanical classification is closely correlated with type of fat. It is not unreasonable therefore to take *N. closterium* fat as typical of most diatom fats, whether marine or freshwater. If this is so, all the freshwater plants—diatoms, algae and higher plants—have fats closely similar to freshwater animal fats and in this case it is possible that the plankton crustacea deposit ingested fat largely unchanged in type. In freshwater some of the larger plant forms also contribute to the animal food, *via* the medium of herbivorous fish, molluscs *etc.*, and here also there is no reason for ingested fat to be modified to any great extent.

SUMMARY.

The proportions of the various fatty acids in a number of algal fats have been examined. The fats fall into groups agreeing with their botanical relationships, and no difference between salt- and fresh-water forms as such could be detected. A marine diatom and a higher aquatic plant both had fats similar to those of green algae.

The results suggest that whilst freshwater plankton crustacea may deposit ingested fat largely unchanged in type, the corresponding marine organisms are forced to modify it considerably.

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