CCXXII. FAT METABOLISM IN FISHES.

VII. THE DEPOT FATS OF CERTAIN FISH FED ON KNOWN DIETS.

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IN a recent paper [Lovern, 1935] the writer has shown that the food fats of most fish are of a similar type to the depot fats of the fish themselves, and that a simple deposition of ingested fat, largely unchanged in composition, would account for the observed compositions of most fish depot fats. Another interpretation (not necessarily exclusive of the first one) will however fit the observed facts equally well. It may be that the whole life of the sea (both animal and vegetable) elaborates the same type of fat, which is thus typically marine, and that fresh-water life also elaborates its own special type of fat. There is abundant evidence moreover that certain fishes and other aquatic animals can modify their food fat very considerably to obtain a depot fat of a highly specialised character. Examples of such are certain elasmobranch fish and the toothed whales. The fact that certain other elasmobranch fish (*e.g.* the skate and thresher shark) and other varieties of whales exhibit no serious difference of type between their depot fats and their food fats may be due only to their own requirements being of the general marine type and not to any inability to modify ingested fat if and when necessary.

It would obviously help in deciding between these two interpretations if information could be obtained as to the type of fat laid down by fish, (*a*) on a diet containing fat of a non-aquatic type only and (*b*) on a fat-free diet. In case (*b*), any fat laid down would presumably have been synthesised from carbohydrate.

With the kind assistance of Mr W. Birtwistle, Director of Fisheries for the Federated Malay States and Straits Settlements, an attempt has been made to investigate these problems. At Singapore a species of carp, *Otenopharyngodon idellus*, is reared in shallow ponds dug in clay, and is fed only on grass. Two varieties of grass are used, elephant grass *Pennisetum purpurascens* (*P. setosum*), and Guinea grass, *Panicum maximum*. The only work on grass fats of which the writer is aware is that of Chibnall and Smith [1932, 1, 2] and Pollard *et al.* [1931] on cocksfoot and perennial rye grasses. From this it was expected that the elephant and Guinea grass fats would not be of the aquatic type.

In addition to these grass-feeding carp, the ponds contain two other carp, *Hypophthalmichthys nobilis* and *H. molitrix*. These fish act as scavengers, eating the faeces of *C. idellus*. In addition they eat the mud from the bottom of the ponds. This mud consists of fine clay reinforced with the faeces of *C. idellus* and with the rotting remains of unconsumed grass. These scavenging fish thus receive a diet almost free from fat (*cf.* p. 1896), but with presumably appreciable carbohydrate formed by bacterial action on rotten grass and in the faeces.

In some of the ponds small quantities of peanut cake are suspended in wire baskets and this is eaten from time to time by *H. nobilis* and *H. molitrix*. None

has ever been found in the stomach of *C. idellus*. The samples of *H. nobilis* and *H. molitrix* examined however had been reared in ponds in which no peanut cake had been suspended.

EXPERIMENTAL.

The grass-eating fish *C. idellus*, in addition to depositing fat in the body muscles, has a considerable quantity of fatty tissue around the intestines. The mud-eating fish has no appreciable quantity of this mesenteric fat.

Samples were sent from Singapore to Aberdeen in various ways. One consignment of *C. idellus* was sent in cold storage, and the body fat and mesenteric fat extracted at Aberdeen (1). A further supply of *C. idellus* flesh was minced at Singapore, desiccated with anhydrous sodium sulphate and sent over in tins, being extracted at Aberdeen. The mesenteric fat from these fish was rendered by simple heating at Singapore, and sent over in a small bottle (2). Later on a further batch of flesh and rendered mesenteric fat was received from *C. idellus* raised in ponds in which peanut cake had never been placed (3). The intestines of the frozen fish were crammed with grass, and it seems fairly certain that in any case this fish consumes no peanut cake.

Supplies of *H. nobilis* and *H. molitrix* flesh were obtained (packed with anhydrous sodium sulphate) from ponds in which no peanut cake had been placed.

Quantities of elephant grass, Guinea grass and the bottom mud of the ponds were also sent over after air-drying and were ether-extracted at Aberdeen. The extracted mesenteric fat from the frozen fish, after storage under nitrogen at -28° for a few months at Aberdeen, had become largely ether-insoluble. The ether-soluble portion contained dibasic acids and it was evident that considerable oxidative breakdown had occurred. The writer has never previously encountered such a case, and presumably during transit from Singapore considerable quantities of peroxide must have been accumulated in the fat. This peroxide must then have decomposed when the extracted fat was stored under nitrogen at Aberdeen. The composition of the remaining ether-soluble portion of the fat is not included below, but it indicated that this mesenteric fat (1) was closely similar in composition to the body fat from the same fish (1). Mesenteric fats (2) and (3) were mixed to give an adequate sample for a full-scale analysis.

In several instances only sufficient fat was available for analysis by semi-micro-methods, and in these cases the results are only expressed to the nearest unit.

The particulars of the fish fats are given in Table I and the fatty acid compositions, as weight percentages, in Table II.

The two grasses proved to contain very little fat. The elephant grass (air-dried, as received) yielded 1.2% of ether-soluble matter and the Guinea grass 0.95%. In each case this was largely non-fatty in nature. In addition to chlorophyll the elephant grass extract contained 40% of unsaponifiable matter and the Guinea grass extract 61.5%. The saponifiable matter was a mixture of glycerides, phosphatides and waxes, which were not separated, as the combined acids were required even for analyses by semi-micro-methods. In any case, it

Table I. *Particulars of fats.*

Species	Sample	I.V.	Sap. equiv.	% un-saponifiable
<i>C. idellus</i>	Body fat (1)	149.7	283.4	2.0
<i>C. idellus</i>	Body fat (3)	118.8	288.9	4.4
<i>C. idellus</i>	Mesenteric fat (2) and (3)	130.0	287.0	0.7
<i>H. molitrix</i>	Body fat	121.2	286.4	2.4
<i>H. nobilis</i>	Body fat	129.3	—	9.5

Table II. *Compositions of fatty acids of carp fats (wt. %).*

Species	Depot	Saturated			Unsaturated				
		C ₁₄	C ₁₆	C ₁₈	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂
<i>C. idellus</i>	Body (1)	1.5	13.6	2.5	1.5	6.7 (-2.0 H)	64.0 (-4.3 H)	10.2 (-6.4 H)	—
<i>C. idellus</i>	Body (3)	2.6	18.0	1.9	0.7	22.9 (-2.1 H)	45.7 (-3.0 H)	8.2 (-6.5 H)	—
<i>C. idellus</i>	Mesentery (2) and (3)	1.9	14.7	2.5	0.2	8.0 (-2.0 H)	64.6 (-3.7 H)	7.6 (-6.0 H)	0.5
<i>H. molitrix</i>	Body	0.8	21.3	1.1	0.6	17.1 (-2.0 H)	49.5 (-3.0 H)	9.6 (-6.7 H)	Trace?
<i>H. nobilis</i> *	Body	—	17.0	6.0	—	9.5 (-2.0 H)	54.0 (-3.6 H)	13.5 (-6.8 H)	—

* Semi-micro-analysis.

Table III. *Compositions of fatty acids from grass fats (wt. %).*

Grass	Saturated						Unsaturated			
	C ₁₀	C ₁₂	C ₁₄	C ₁₆	C ₁₈	C _{>18}	C ₁₄	C ₁₆	C ₁₈	C _{>18}
<i>Penn. purpurascens</i> *	Trace	2	1	9	3	14	2	12 (-2.0 H)	25 (-4.0 H)	32 (-2 H)
<i>Pan. maximum</i> *	—	7	1	11	7	10	2	15 (-2.5 H)	27 (-4.1 H)	20 (-2 H)

* Traces of lauroleic and arachidic acids present.

was knowledge of the total dietary fatty acids that was needed. The compositions of the mixed fatty acids are given in Table III as weight percentages.

The C_{>18} acids were presumably derived from the wax fractions. Both saturated and unsaturated acids contained at least 26 carbon atoms but were undoubtedly mixtures which were not further dealt with. The dried mud contained 0.17 % of ether-soluble material. The major portion of this was insoluble in light petroleum and therefore could not be true fat. The petroleum-soluble material had i.v. 57.5, and contained 57 % of unsaponifiable matter. The acids recovered after saponification were again largely insoluble in light petroleum and the final yield of true fatty acids was 0.007 % of the weight of mud. These fatty acids were of relatively high melting point, had i.v. only 50.0 and gave no ether-insoluble bromides. They appeared to contain some of the wax acids, which would be expected in the faeces of *C. idellus*, and obviously contained no polyunsaturated C₂₀ acids.

DISCUSSION.

It had been anticipated that the grass fats would be of simpler composition than is actually the case, and that apart from wax acids the fatty acids would be almost entirely palmitic and C₁₈ unsaturated acids. The occurrence of palmitoleic acid (and myristoleic acid) in these grasses is interesting, as they have only been found so far as plants are concerned in algae and other aquatic plants [Lovern, unpublished observations].

It must be remembered however (as mentioned by Tsujimoto [1925]) that most of the detailed work on plant fats has been limited to seed and fruit fats, whereas the work on aquatic plants (and these grasses) has involved the leaves and stalks of the higher plants and the whole thallus of the algae.

In view of the occurrence of these "aquatic" acids in the grasses, it is not certain that *C. idellus* is able to synthesise these acids from carbohydrate sources.

This fish also contained C_{20} polyethylenic acids, but the grass fats may well have contained small quantities of such acids, which were inseparable (with the small quantities of material available) from the unsaturated wax acids. It should be noted however that the proportion of available fatty acids in the grass diet is very low. Leaving out of account the unsaponifiable matter and the wax acids (which would not be assimilated), the elephant grass contains only 0.34 % and the Guinea grass 0.17 % of available fatty acids on the dry material. *C. idellus* contained a considerable proportion of fat to total body weight (3.7 % in muscles (wet weight) in addition to the mesenteric fat). Whilst a slow accumulation of ingested fatty acids (together with a readjustment of the relative proportions of these) could account for the observed compositions of the fish fats, the available evidence, in the writer's opinion, is rather in favour of a synthesis by the fish, from carbohydrate or other material, of fatty acids of the desired type.

This view is strengthened when the results from the mud-eating fish are considered. The mud itself furnished a diet to all intents and purposes fat-free, and even the 0.007 % of fatty acids which it did contain was free from polyethylenic C_{20} acids, whilst the fish eating this mud contained appreciable amounts of these acids in their fat. The only other source of ingested fat would be the faeces of *C. idellus* consumed directly. The mud would contain an appreciable quantity of these however and if the faecal fat had been considerable in quantity and of the "aquatic" type it should have been detected in the mud fat. Further, if *C. idellus* excretes any appreciable proportion of "aquatic" fatty acids in the faeces, this would render it almost certain that the fish synthesised its own depot fats, in view of the almost fat-free nature of the grass diet.

SUMMARY.

The depot fats from three species of tropical carp have been analysed. The first species was raised on a diet consisting only of grass, whilst the other two species lived on mud in which the organic matter consisted of the faeces of the first species together with rotting grass leavings.

Two species of grass were used, and both contained only small quantities of fat. This fat was found to be of an unexpectedly complete type and contained acids formerly encountered only in aquatic plants. The mud furnished a fat-free diet.

Whilst the complex nature of the grass fats makes the interpretation of the results less certain, the evidence taken as a whole indicates that these fish are able to synthesise fat of the desired type from carbohydrate or other sources.

REFERENCES.

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