9) were found to have more double bonds below than above the 9-position. This does not necessarily mean that the predominant direction of migration was toward the ester linkage. As the distance of a double bond from the ester linkage increases, its rate of hydrogenation increases (15). Hence a sizable proportion of double bonds which migrated to the outer positions may have been preferentially hydrogenated and did not appear in the end product.

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Biogenesis of Polyunsaturated Acid in Fish[1]

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IT HAS BEEN KNOWN for many years that the fatty acids of fish are more highly unsaturated and have greater average chain-lengths than those of most mammals. That the differences may be even more basic became apparent as the structures of the fatty acids were determined. Despite their high unsaturation fish oils, in general, did not serve as a source of essential fatty acids (1). This would indicate that they do not belong to the linoleic family

$$[CH_3(CH_2)_4 - CH = \text{termination}]$$

(2,3) as do most of the mammalian polyunsaturated acids. Structure determinations of individual fatty acids have confirmed these ideas. For example, Klenk and his coworkers (4,5) have shown that most of the polyunsaturated fatty acids of herring oil belong to the linolenic family ($CH_3 - CH_2 - CH =$ termination) (2,3), a finding which appears to agree with those of Stoffel, Insull, and Ahrens (6) for menhaden oil.

If the fatty acids of fish are so different from those of mammals, the question of their derivation and transformations is of interest. It has been shown in these laboratories (3) that the synthesis of polyunsaturated fatty acids in the rat (and presumably other mammals) is accomplished by the addition of double bonds in the divinyl methane relationship to unsaturated fatty acids derived from the diet or synthesized in the animal body. In mammals the parent acid is usually linoleic from the diet, thus leading to the family of essential fatty acids including γ-linolenic, 8,11,14-eicosatrienoic, and arachidonic acids. In the absence of linoleic acid, dietary linolenic and biosynthetic oleic and palmitoleic acids can also be converted to higher polyunsaturated acids. The problems to be considered in this connection are whether the polyunsaturated acids of the fish are predominantly of the linolenic family because the fish, unlike the mammal, can synthesize their precursor or whether they are formed by the familiar process from dietary fatty acids largely of the linolenic type. The possibility that fish possess, to a greater degree than mammals, the ability to extend oleic acid must also be considered.

Klenk (personal communication) found that, following the injections of acetic-1-C^{14} acid into fish, ozonolytic degradation of their polyunsaturated acids acids revealed some activity in the malonic acid fraction. This could only have arisen by a synthetic process, but the nature of the fatty acids degraded was uncertain. Reiser and his coworkers (7,8) found that the nature of the fish fatty acids is markedly influenced by diet and came to the conclusion that although the differences between the fatty acid compositions of marine- and fresh-water fish are due to differences in their diets, they also have a marked ability to synthesize polyunsaturated but not necessarily essential fatty acids. When these authors placed fish on a fat-free diet, their polyunsaturated fatty acids were reduced but they did not appear to develop any deficiency symptoms. This would be expected from the results of similar treatment of adult rats.

In the present experiment an attempt was made to ascertain which, if any, polyunsaturated acids might be synthesized by fish and to study further the effect of dietary fat on their deposited fatty acids.

Experimental

Treatment of Animals. Three mature female *Tilapia mossambica* which had been raised in salt water and

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three which had been raised in fresh water, all on Purina Trout Chow, were maintained on ether-extracted Purina Trout Chow for five days. The fish were then each given an intraperitoneal injection of sodium acetate-1-C^{14} for a total of 0.5 mc. After 6 hrs. they were killed by freezing.

Extraction and Separation of Lipids. The fish were extracted in a Waring Blendor with alcohol, alcohol: ether (70:30), and ether. Evaporation of these solvents and drying of the residue *in vacuo* gave the total fat. Weights of these fractions are listed in Table I.

TABLE I

Weights of Fractions of Salt- and Fresh-Water Fish

	Total wt. fish	Fat-free residue	Total lipid
	g.	*g.*	*g.*
Salt water	288	76.5 (26.6%)	2.172 (0.75%)
Fresh water	490	134.5 (27.4%)	3.255 (0.66%)

There appears to be no significant difference between salt- and fresh-water fish in these respects.

The lipid of the two groups was combined and saponified with alcoholic potassium hydroxide, and the fatty acids thus obtained were separated by low-temperature crystallization from acetone. Weights and activities of these fractions are listed in Table II.

TABLE II

Weights and Activities of Fatty Acid Fractions

Fraction	Weight	Activity
	g.	*(d.p.s. per mg.)*
Total fatty acids	3.82	600
Saturated fatty acids (insol. at −5°C.)	0.52 (15.5%)	695
"Mono-unsaturated" fatty acids (insoluble at −50°C.)	1.69 (50.1%)	297
"Polyunsaturated" fatty acids (soluble at −50°C.)	1.16 (34.4%)	94

The mono-unsaturated and polyunsaturated fractions were analyzed on the gas-liquid chromatogram.[5] In Table III are given the approximate compositions of these fractions, as calculated from the chromatograms.

Since the "polyunsaturated" fraction, which had the desired polyunsaturated acids also contained considerable quantities of oleic acid, which might interfere with further work, a separation was made on the reversed-phase column as described previously (10). That the oleic acid was completely removed by this process was confirmed by gas chromatographic analysis as described above. A sample of the polyunsaturated fraction thus obtained was methylated with diazomethane and separated on a preparative gas chromatographic column.[5] From 69 mg. of the mixed methyl esters was obtained: 4.3 mg. methyl palmitoleate (261 d.p.s. per mg.), 5.8 mg. of methyl linoleate (33 d.p.s. per mg.), and 7.7 mg. of methyl arachidonate (60 d.p.s. per mg.). Other fractions were not collected.

Degradation of Acids. Two types of degradation were used in these studies: stepwise degradation, using the method of Dauben, Hoerger, and Petersen (11) modified as described previously (12), and permanganate oxidation, essentially as described by

[5] Analyses were performed, using a Wheelco Model 10 (Barber-Colman Company) with a 6-ft. 6-mm. I.D. column of ethylene glycol-succinate polyester on 60–100 mesh celite at 178°. Preparative separations were carried out, using the Aerograph Model A-100-C (Wilkens Instrument and Research Company Inc.) using a 5-ft. ½-in. column of Craig Polyester-succinate (1,4-butanediol-succinate polymer).

TABLE III

Composition of Unsaturated and Polyunsaturated Fatty Acid Fractions

Acid		% of fraction	% total "unsaturated" fractions	% of total fatty acids[a]
Saturated		.....	.....	30.2
"Mono-unsaturated" fraction				
Myristic	(14:0)[b]	3.6	2.5	.....
	(14:1?)	3.7	2.6	.....
Palmitic	(16:0)	31.5	18.7	.....
Palmitoleic	(16:1)	3.8	5.1	4.3
Stearic	(18:0)	20.7	12.3	.....
Oleic	(18:1)	28.0	23.6	19.9
Linoleic	(18:2)	8.6	11.2	9.5
"Polyunsaturated" fraction				
Myristic	(14:0)	0.8	.....	.....
	(14:1)	1.8	0.7	0.6
Palmitoleic	(16:1)	9.5	.....	.....
	(16:2)	2.6	1.0	0.9
Oleic	(18:1)	17.0	.....	.....
Linoleic	(18:2)	15.0	.....	.....
	(18:3)	4.5	1.8	1.5
	(20:3)	Trace	.....	.....
Arachidonic	(20:4)	13.4	5.5	4.7
Unknown A	(22:4?)	3.1	1.3	1.1
Unknown B	(22:5?)	6.7	2.7	2.3
Unknown C	(22:6?)	25.4	10.3	8.7

[a] Assuming the fraction insoluble at −5°C. to contain only saturated acids.
[b] Shorthand designation (9).

Haverkamp-Begemann, Keppler, and Boekenoogen (13).

The methyl arachidonate obtained as described above was diluted to 250 mg. with pure unlabelled methyl arachidonate[6] and was hydrogenated over palladium on charcoal and saponified to give 228 mg. of arachidic acid with 2.1 d.p.s. per mg. Gas chromatographic analysis of a sample of the methyl ester revealed a single peak. The arachidic acid (220 mg.) was degraded stepwise (11,12) to give stearic acid and 2 molecules of benzoic acid, representing carbons 1 and 2 of the original arachidonic acid. The activities of these acids are listed in Table IV.

TABLE IV

Activities of Degradation Products of Arachidic Acid

Acid	Activity
	(d.p.s. per m. mole)
Arachidic acid	664
Nonadecanoic acid	152
Octadecanoic acid	165
Benzoic acid (C_1)	521
Benzoic acid (C_2)	32

The percentage activity of the various carbons of the arachidic acid is as follows:

$$\underset{23}{C_{18}H_{37}} - \underset{6}{CH_2} - \underset{71}{COOH}$$

The methyl linoleate was diluted with inactive methyl linoleate to give 121 mg. with activity of 1.7 d.p.s. per mg. After saponification a sample of 68.5 mg. was oxidized with 316 mg. of potassium permanganate in acetic acid (13,14). Decolorization of the reaction mixture with formic acid, followed by dilution and extraction with pentane, yielded caproic acid with an activity of 1.4 d.p.s. per mg. Repeated extraction of the aqueous residue with ether gave azelaic acid with an activity of 1.4 d.p.s. per mg.

Preparation of Tetrabromostearic Acid. A diluted sample of the above prepared linoleic acid (21.0 mg., 0.95 d.p.s. per mg.) was brominated in ether with 33 mg. of Br_2. After evaporation of the ether the pen-

[6] Obtained from the Hormel Institute.

tane-insoluble solid was crystallized twice from 82% of acetone. The resulting tetrabromostearic acid had an activity of 0.074 d.p.s. per mg. (expressed on the basis of the bromine-free compound). Too little activity remained for further purification.

Discussion

The fish employed in these studies were maintained on Purina Trout Chow until the start of the experiment when they were fed the same diet after ether extraction. Under these circumstances the fish deposited fat which apparently contained high proportions of the typical mammalian polyunsaturated acids, linoleic and arachidonic acids. Linolenic acid, the typical marine fatty acid, is present in very small amounts. It therefore appears in agreement with previous studies (7,8) that the fish deposits, to a significant extent, the fatty acids of the diet. In this case these were the typical animal and vegetable fatty acids furnished by the 4% of fat in the diet.

The longer-chain, more highly unsaturated acids, for which arachidonic may be taken as an example, appear to be formed by desaturation and elongation processes typical of the mammalian metabolism (3). If this is indeed the case, structure determination will probably show them to be derived largely from oleic or linoleic acid and not from linolenic acid as is the case with fish on a natural marine diet (4,5). Thus the same processes of alteration of unsaturated fatty acids found in mammals probably holds for fish with the exception that in the latter case the process continues to produce fatty acids of somewhat greater chain-length and higher degree of unsaturation.

The tracer studies revealed that under conditions of the experiment the fish synthesized large amounts of fatty acids. In agreement with animal studies the rapidly synthesized fatty acids were largely of the saturated type; the polyunsaturated acids were by far the least active.

Degradation studies indicated that the 20-carbon polyunsaturated acid, probably largely arachidonic, was synthesized by addition of acetate to a relatively inactive 18-carbon portion, probably largely derived from linoleic. The nature of the active 18-carbon acid that contributed activity to the linoleic and the terminal 18 carbons of the arachidonic acid was indicated by two experiments. Degradation of the linoleic fraction showed it to contain equal activity in the carboxy and methyl moieties. That the active acid in the fraction was not linoleic is revealed by the fact that the activity did not appear in the recrystallized tetrabromostearic acid derived from the linoleic acid. A suggestion as to its nature can be made. Since it had uniform activity throughout the molecule, it could have been derived from oleic acid (similar degradation of the crude oleic acid fraction from these fish revealed that, unlike mammals, fish appear to synthesize uniformly labelled oleic acid). Furthermore an octadecadienoic acid derived from oleic must be postulated for rats which have been fed a fat-free diet (14). This is the 6,9-octadecadienoic acid, which is a probable intermediate between oleic and 5,8,11-eicosatrienoic acids (14). Another probability is the postulated 8,11-octadecadienoic acid intermediate between palmitoleic and 7,10,13-eicosatrienoic acids (14). The existence of this acid in rats has been demonstrated by Fulco and Mead (3). Either of these acids would have the properties of the active octadecadienoic acid postulated in the present study.

In any event the tracer studies tend to confirm the hypothesis that polyunsaturated fatty acid metabolism is not qualitatively different in fish and mammals. Although small quantities of polyunsaturated acids can be synthesized from acetate, these are apparently not the higher essential fatty acids, which must be formed from dietary linoleic acid.

Summary

Following injection of *Tilapia mossambica* with acetate-1-C^{14}, their fatty acids were isolated, fractionated, and degraded. The high content of linoleic and arachidonic acids was evidently derived from the diet. Degradation of these acids revealed a distribution of carbon-14 similar to that found in similar studies on mammals.

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Search for New Industrial Oils. IV.

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As related in previous papers of this series (1), a program is in progress to determine by chemical screening analyses what amounts and general classes of fatty acids are contained in seed oils of a large number and variety of presently uncultivated species. Those with suitably high oil content, and with fatty acid composition thought to be sufficiently different from that of present commercial vegetable oils to make them of potential practical interest, are then scheduled for more intensive chemical study.

In this paper we report results obtained on 158 species representing 52 plant families in 23 orders. Of these, 138 are previously unreported in the compilations of Hilditch (2) and Eckey (3) or in more

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