liter of falling hypophase revealed that the early fractions were highest in linolenate content (Table II). No contaminant was detectable in the first five samples. However, it may be that means of detection more sensitive than those employed would yield evidence of trace impurities. The upper limit of solubility of linolenate in the aqueous medium was near 0.3% (1.3% on the basis of derivative wt). A product (15.81 g) of purity above 99% methyl linolenate which represented 70% of the total amount isolated in the experiment was recovered in the first 6 liters; of the linolenate originally present 22.47 g or 89.8% was removed in 12 liters. In a typical bromination-debromination procedure (7) about 10% of the linolenic acid in the starting linseed oil is isolated.

Isolation of a Polyunsaturated Fraction from Fish Oil. A (9 g) clear liquid concentrate of highly unsaturated esters (iodine value 395, n_{40}^{D} 1.4821) was obtained from 50 g of the mixed methyl esters of pilchard oil by the mercuric acetate procedure. The first 4 liters of 10% methanol to pass through the ether layer carried more than 90% of this material. After alkali isomerization of a portion of the concentrate for 15 min with 21% KOH-glycol absorptivity values at 346μ and 315μ were 33.0 and 44.5, respectively. Unfortunately, the absorbance of the isomerized sample was not read at 375μ to obtain data on hexene.

TABLE II

Fractionation of Mercurated Methyl Esters of Linseed Oil

Fraction[a]	Weight	Composition[b]		
		Linolenate	Linoleate	Oleate
	g	%	%	%
Original[c]	50.13	51.0	15.7	22.1
1	2.83	100	.....	.....
2	2.43	100	.....	.....
3	2.92	100	.....	.....
4	2.86	100	.....	.....
5	2.41	100	.....	.....
6	2.36	99.1	0.9	.....
7	2.01	97.8	1.6	0.6
8	1.36	99.5	0.5	.....
9	1.20	98.4	1.6	.....
10	.87	98.0	2.0	.....
11	.65	95.7	3.2	1.1
12	.58	95.2	3.9	0.9
Final[c]	24.85	8.7	30.1	43.7

[a] Each of the fractions 1–12 was extracted from the ether epiphase with 1 liter of 10% aqueous methanol which had been equilibrated with ether.

[b] The composition in terms of weight % was calculated from the gas chromatographic peak areas determined by multiplying the peak height × the width at half-height.

[c] The original mixed esters contained 1.4% palmitate and 3.8% stearate; the residual esters contained 11.7% palmitate and 5.8% stearate.

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• *Letters to the Editor*

Evidences for Fat Secretion in the Intestine of the Fish

IN THE COURSE of an experiment concerning the "in vivo" incorporation of C^{14}-labeled acetate into fish fatty acids, the intestinal and body fat from a group of fish kept on a fat free diet was studied. Two fresh water fish (*Pimelodus maculatus*, Bagre) were fed a fat-free ration similar to that described by Kelly et al. (1) except that fish meal (VioBin Corp., Ill.) was substituted for egg albumen. Both the starch and fish meal in the diet were extracted with acetone. The fat content of the entire diet was determined to be 0.2 g/100 g. After 10 weeks on that ration the fish were injected intraperitoneally with 0.18 mc/kg of body wt of sodium 1-C^{14}-acetate. The fish were sacrificed 24 hr later and their muscle and liver lipids were first extracted by the method of Folch et al. (2) and then fractionated on silicic acid into triglycerides and phospholipids. The lipids in the intestinal lumen were washed out according to the procedure of Irwin et al. (3) and Deuel et al. (4). Fatty acid compositions were ascertained by gas liquid chromatography on a column of 10% ethylene glycol adipate polyester on chromosorb P (Johns-Manville, New York) using argon as the carrier gas. Fat samples mounted on frosted glass planchets were tested for radioactivity in a GM counter, Model D-47 (Nuclear Chicago Corp., Ill.) with a "Micromil" window, up to 10,000 counts.

The lipids collected from the intestines amounted to 16 and 25 mg, respectively. The fact that they were radioactive proved unequivocally that, as it has been demonstrated in the rat (5), fat is secreted into the intestinal lumen of the fish. The fatty acid com-

Fatty Acid Composition of Muscle Triglycerides and Intestinal Lipids of Fat Free Diet Fed Fish (in moles %)[a]

Fatty acid[b]	Muscle triglycerides	Intestinal lipids	Fatty acid[b]	Muscle triglycerides	Intestinal lipids
12 to 14	1.8	3.4	20:0	tr.	4.1
15:0 r[c]	0.8	1.5	20:1	1.8	tr.
15:0	0.6	5.4	20:2 ?	0.1	tr.
16:0 r[c]	0.2	0.2	20:3 ?	0.3	0.3
16:0	20.0	15.5	20:3' ?	0.2	0.4
16:1	6.7	3.6	21:0	.....	2.3
16:2 or 17:0 r[c]	0.6	0.7	20:4	0.5	tr.
17:0	0.4	5.3	20:5	0.2	0.4
16:3 or 17:1 or 18:0 n or c[d]	0.4	.....	22:0	tr.	.....
			22:1	tr.	.....
18:0	7.3	5.0	22:4 ?	tr.	0.5
18:1	54.2	43.8	22:5 ?	0.2	0.3
18:2	2.8	1.3	22:5'	tr.	0.3
18:3	0.2	.....	22:6	0.3	0.7
19:0	tr.	4.5			
19:1 ?	0.2	0.3			
18:4 ?	0.2	0.2			

[a] Fatty acid composition of liver triglycerides was very similar to that of muscle triglycerides and consequently not shown.

[b] The number before the colon stands for the number of carbons in the chain. The number after the colon indicates number of double bonds.

[c] r = iso acids.

[d] n or c stands for neo or cyclic acids.

position of the intestinal lipids showed, when compared to that of muscle and liver triglycerides (see Table), a strong similarity, in spite of the presence in the intestine of around 20% of odd-chain fatty acids. The view is advanced that these odd-chain fatty acids could be brought about by the action of the intestinal bacteria. The differences found among the specific radioactivity levels of the intestinal lipids (36 cpm/mg), muscle triglycerides (11 cpm/mg), and liver triglycerides (91 cpm/mg) would indicate that the intestinal lipids do not arise directly from muscle or liver lipids, but from a pool constituted by fats from various sources as could be the blood lipids.

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Charring Conditions for the Quantitative Analysis of Mono-, Di-, and Triglycerides by Thin-Layer Chromatography

IN OUR METHOD for the quantitative analysis of mono-, di-, and triglycerides by TLC (2) it was observed that equal amounts of these compounds gave spots of different intensities on charring far out of proportion to the differences in their carbon densities. Furthermore, unsaturated glycerides gave much darker spots than saturated glycerides. These observations made it necessary to recommend the use of pure reference compounds as standards for the TLC analysis of mono-, di-, and triglycerides. For precise results, it was recommended further that the sample be hydrogenated prior to analysis so that saturated reference compounds could be used.

When the charring process is carried out by heating the chromatoplates on a hot plate (up to 360C) after spraying them with 50% aqueous sulfuric acid as commonly practiced (2), the amount of char (yield of carbon) in each spot represents a balance between two processes: evaporation and oxidation.

Saturated glycerides give less intense spots than unsaturated glycerides because, being more resistant to oxidation, more evaporation can occur in these compounds prior to their oxidation to carbon. Since there is a great difference in the volatility of mono-, di-, and triglycerides, the differences in the intensity of the charred spots of equal amounts of these compounds can be explained on the basis of the relative amount of evaporation that occurs prior to their oxidation to carbon.

TABLE I

TLC Analysis of Mono-, Di, and Tripalmitin *

	Known (% wt)	Found	
		180C (chromic-sulfuric)	260C (sulfuric)
Monopalmitin	32.0	33.3	26.1
Dipalmitin	35.6	33.0	33.6
Tripalmitin	32.4	33.7	40.3

* Determined on separate chromatoplates at an R_f value of approximately 0.5 (2).

When the charring is carried out below 200C and with a more powerful oxidizing agent than 50% aqueous H_2SO_4 such as chromic-sulfuric acid (1), the amount of evaporation prior to charring is insignificant. Mono-, di-, and triglycerides give spots of equal intensities after correction for differences in their carbon densities (Table I). Unsaturation, *per sec*, also has no effect on the yield of carbon under these conditions of charring as demonstrated by the analyses of tripalmitin and triolein (Table II).

TABLE II

Densitometer Analysis of Tripalmitin and Triolein at 180C with Chromic-Sulfuric Acid

Wt of sample	Tripalmitin,* peak area		Triolein, peak area
(μg)			
2.0	22.0	(10.7)	22.5
5.0	45.0	(23.8)	50.0
10.0	102	(50.4)	106
15.0	137	(65.0)	137
20.0	182	(86.4)	182

* Results in brackets on tripalmitin were obtained by charring the spots on a hot plate with 50% H_2SO_4 (2).

The spray reagent used in the above analyses consisted of a saturated solution of $K_2CR_2O_7$ in 80% (by wt) H_2SO_4. The chromatoplates are sprayed lightly with the reagent, then heated at 180C for 25 min.

It should be noted that separate solvent systems are used for the analysis of mono-, di-, and triglycerides (2) in order that the analysis of each component may be made at approximately the same R_f value.

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