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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