ships of our observations to the hypoxia theory of carcinogenesis and to tumor radiosensitivity are discussed.

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Intestinal Absorption of Unhydrolyzed Tripalmitin.* (22572)

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One of the oldest and most important questions on the mechanism of fat absorption is the degree of absorption, if any, of unhydrolyzed triglycerides(1). Several years ago this laboratory published results of a study of absorption of C^{14} glycerol labeled conjugated trilinolein(2). Only about 60% of the labeled glycerol appeared in the lymph fat. It was concluded, therefore, that at least 40% of ingested triglycerides were completely hydrolyzed. The relative amounts of labeled glycerol in the saturated and unsaturated triglycerides of lymph fat after ingestion of mixtures of labeled fat with unlabeled saturated triglyceride, led to the conclusion that the remaining 60% of the ingested triglycerides were absorbed as monoglycerides.

The design of the above experiment was such that the absorption of relatively small amounts of unhydrolyzed triglycerides could not have been detected. The present study is an effort to determine more accurately if small amounts of unhydrolyzed triglycerides may be absorbed.

Methods. If a mixture of 10% saturated triglyceride and 90% unsaturated triglyceride are completely hydrolyzed and resynthesized by random distribution, the resultant fat will contain 0.1% saturated triglycerides. Any saturated triglycerides in the resultant fat above 0.1% will be equal to the quantity of

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the original which escapes hydrolysis. This principle was used to test the degree of absorption of unhydrolyzed tripalmitin by the measurement of labeled tripalmitin in thoracic duct chyle after ingestion of a mixture of 10% labeled tripalmitin, and 90% triunsaturated triglyceride. *Collection of lymph.* A 200 g white rat was fasted 24 hours and its thoracic duct cannulated at 2 p.m. After 10 hours the animal was offered one-half g of fat free synthetic ration plus a mixture of 20 mg of tripalmitin, C^{14} labeled in both glycerol and fatty acid moieties, and 180 mg of a triunsaturated glyceride.‡ The feed was promptly and completely consumed. The lymph became milky after about 20 minutes. Fifty ml of lymph was collected during the following 9-hour period, when it was quite clear. At 9:15 a.m. the rat was given a second meal containing 20 mg of doubly labeled tripalmitin and 180 mg of triunsaturated glyceride. The lymph again became cloudy in about 15 or 20 minutes. At 3 p.m., after lymph had become clear, lymph collection was discontinued. Approximately 50 ml were collected during the second period. The rat drank freely of 0.9% saline during lymph collection. *Isolation of saturated triglycerides.* The lymph collected during each period was diluted with alcohol, heated to denature the protein, and evaporated to dryness under reduced pressure. The dry residue was extracted with several small volumes of chloroform. The clear, dry chloroform solution was passed through a 10 g column of 100 mesh silicic acid to remove phospholipides(3). The chloroform solution of neutral fat and washings were pooled, evaporated to dryness, and weighed. During the first period 247 mg of fat were recovered from the lymph and 186 mg during the second period. The fat from each period was dissolved in 6 ml of acetone and the solution held at 25°C overnight. No crystals of saturated triglycerides appeared. It was probable that some saturated triglyceride was present but in too small quantity to be isolated by this method. Therefore, 125 mg of unlabeled tripalmitin was added as carrier and the solutions held at 8°C overnight and filtered(4). The precipitates were dissolved in another 6 ml of acetone and the crystallization repeated, but only for 2 hours. To wash out any unsaturated triglycerides containing labeled glycerol, 2 drops of triunsaturated triglycerides were added to the precipitates and the mixtures again crystallized from 6 ml of acetone. The crystals insoluble at 8°C were finally crystallized from 6 ml of acetone at 26°C overnight. The insoluble crystals were dried and weighed. From the first period lymph 122 mg of saturated triglycerides were obtained, and 119 mg from the second.

Results. Percentage of ingested palmitic acid recovered in the lymph. To estimate the percentage of ingested labeled palmitic acid which appeared in the lymph, the acetone soluble and insoluble fractions of lymph neutral fat of each period were saponified and the fatty acids weighed. The acids were dissolved in 10 ml of chloroform and volumes containing approximately 0.3 mg evaporated on planchettes and the specific activities determined. A similar determination was made of fatty acid of the labeled tripalmitin fed. Percentage of ingested labeled fatty acids which appeared in the lymph could then be calculated from the specific activities and weights of ingested and lymph acids. (Cts/min/mg x mg fatty acid = total cts/min. Total cts/min of lymph acids ÷ total cts/min ingested acids x 100 = percentage of ingested acids in the lymph.) The data are given in Table I.

Percentage of tripalmitin absorbed unhydrolyzed. Glycerol in saponification liquors of the lymph saturated triglycerides and ingested fat were oxidized with periodate and the resultant formaldehyde precipitated with dimethyldihydroresorcinol(2). The activity was corrected to infinite thinness. Since 4.8% and 3.8% of labeled fatty acids of lymph occurred in the saturated triglycerides, one might conclude, as a first approximation, that these quantities of labeled tripalmitin were absorbed unhydrolyzed. However, the ratio of specific activity of fatty acids to that of glycerol is 1.63 in the tripalmitin fed, but 2.23 and 2.13 in the saturated triglycerides of lymph fats of periods 1 and 2. This loss of

‡ Prepared from the unsaturated fatty acids of cottonseed oil.

TABLE I. Ingested Labeled Fatty Acids in the Lymph Fat.

Period	Ingested fatty acids			Lymph fatty acids						Lymph counts / Ingested counts × 100†
				Acetone soluble		Acetone insoluble				
	mg‡	C/m/mg*	Counts	mg	C/m/mg*	Counts	mg	C/m/mg*	Counts	% of total	Total counts	
1	17.7	9540	168858	308	393	120044	107	55.8	6026	4.8	126070	75
2	17.7	9540	168858	145	655	95975	109	34.2	3728	3.8	99703	59

* Counts/min./mg.
† These values are equal to percentages of ingested labeled fatty acids recovered from the lymph.
‡ Labeled polmitic acid from 20 mg of labeled tripalmitin.

active glycerol indicates some synthesis of lymph saturated triglycerides from hydrolyzed labeled fatty acids and unlabeled glycerol. For this reason the labeled glycerol in the saturated lymph triglycerides, and not the acids, should be used as a measure of triglyceride absorption.

From Table II it may be seen that the total glycerol activity ingested was 84215 counts per minute. In the first period 75% of ingested fatty acids appeared in the lymph. If the entire 75% had been absorbed as unhydrolyzed tripalmitin, the total glycerol activity of lymph saturated triglycerides would have been 63161 counts per minute. But only 2142 counts per minute or 3.3% of this theoretical amount was present. Therefore, 3.3% of the absorbed fat was unhydrolyzed.

In period 2, 3.2% of the absorbed fat was unhydrolyzed.

Discussion. The values of 3.3% and 3.2% of ingested tripalmitin which were concluded in the present study to have been absorbed unhydrolyzed, are the upper limits and probably too high. It has been demonstrated that about 60% of ingested fat is absorbed as monoglycerides(2). Resynthesis of these monoglycerides to triglycerides in the intestinal mucosa, in the present experiment, would result in about 0.06% labeled tripalmitin in lymph fat. The values of 3.3 and 3.2% are thus too high by at least 0.06%.

It is assumed in this and in earlier calculations(2) that if any triglyceride is absorbed, it does so without hydrolysis and resynthesis in the lumen of the intestine. Borgström has repeatedly(5-7) challenged this assumption. He has shown that in the lumen of the intestine as well as *in vitro,* pancreatic lipase is capable of resynthesizing triglycerides from monoglycerides and fatty acids. Theoretically, therefore, the assumption is false. Nevertheless, this factor can only invalidate the conclusions reached by the type of study herein described if the amount of rearranged triglycerides absorbed from the lumen is a significant fraction of the total. In Borgström's studies(6) 1.7 mg of labeled palmitic acid was fed in 1000 mg (roughly) of oil. Even at this very low ratio only 20% of free

TABLE II. Absorbed Unhydrolyzed Tripalmitin.

Period	Ingested tripalmitin glycerol* mg	C/m/mg	Cts/m	Lymph tripalmitin glycerol* mg†	C/m/mg	Cts/m	Cts/m‡	Absorbed unhydrolyzed tripalmitin, %
1	14.47	5820	84215	90.5	23.2	2100	63161	3.3
2	14.47	5820	84215	90.5	17.3	1566	49478	3.2

* As methylene bismethone.

† Calculated from the 125 mg of tripalmitin added to precipitate the traces of lymph tripalmitin.

‡ Theoretical value if all absorbed fat had been unhydrolyzed.

fatty acids were incorporated into intraluminary triglycerides. At the most (one molecule of fatty acid per molecule of fat) this could constitute only 0.1% of the intraluminary fat. At higher proportions of free fatty acids the percentage of incorporation should be expected to be even less. Borgström's studies are thus not designed to test the *degree* of randomization of intraluminary triglycerides. Other considerations, such as slow emptying time of fat from the stomach, rapid absorption of monoglycerides and free fatty acids and slow reaction time of lipase in the synthesis of triglycerides, are convincing arguments that the degree of such randomization must be low.

Summary and conclusion. A mixture of 20 mg of tripalmitin, C^{14} labeled in both the glycerol and acid moieties, and 180 mg of triunsatured triglycerides, was mixed in 1 g of a fat free ration and fed to a rat with a thoracic duct cannula. Lymph was collected for 9 hours. A second meal was fed and the lymph collected for 6 hours. It was found that in the 2 periods respectively, 3.3 and 3.2% as much of the labeled glycerol appeared in the lymph saturated triglyceride as would have appeared there had all the labeled lymph acid been absorbed unhydrolyzed. It was concluded, therefore, that only 3.3 and 3.2% of the tripalmitin was absorbed unhydrolyzed.

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