paper chromatography (phenol saturated with water) when N-acetylglucosamine appeared as the only substance giving a positive reaction with the Elson and Morgan spray reagent[6].

The results shown in Fig. 3 indicate that the release of N-acetylglucosamine from the chitin suspension stopped while the decrease in turbidity was still taking place. It therefore appears that possibly here, as in *Streptomyces griseus*[4], chitin breakdown may involve several enzymes, one of which causes depolymerization of the chitin chains, resulting in a lowering of the turbidities of chitin suspensions, another enzyme, which appears to be slowly inactivated at 37°, being responsible for the release of N-acetylglucosamine, either from the polysaccharides originally present, or from oligosaccharides produced enzymically. De-acetylation of the substrates was not observed.

Solutions of the *Carcinus* chitinase system have been used to demonstrate the release of N-acetylglucosamine from chitin samples obtained from several crustacean species by both the extraction method of BRACH[3], and the milder procedures of FOSTER AND HACKMAN[7].

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Pathways of triglyceride formation in adipose tissue

The assimilation of fatty acids by adipose tissue takes place predominantly by transformation into triglycerides, and the rate of uptake of the acids from the medium was found to be correlated with the esterifying capacity of the tissue[1]. The following reactions may contribute to the incorporation of fatty acids into triglycerides: (a) The formation of an acyl-CoA compound, which then interacts with α-glycerophosphate and finally is converted into triglycerides by a series of reaction steps, demonstrated in liver tissue[2,3]. The presence of the enzymes for this reaction sequence in adipose tissue has been demonstrated[4]. (b) The acyl-CoA compound formed may interact with diglycerides present in the tissue to form directly a triglyceride. (c) A lipase-activated exchange reaction between triglycerides and free fatty acids may introduce some of the radioactive fatty acid into the triglyceride[5].

It was of interest to determine which of these three possibilities was the dominant

Abbreviation: CoA, coenzyme A.

one, since this knowledge may be relevant for an understanding of the assimilation of fatty acids by adipose tissue, and of the nature of the acceptor for the fatty acids.

When radioactive palmitate is incorporated into the triglycerides, it is possible to differentiate between the first of these three alternatives and the other two, since with the last (b and c), only one fatty acid in the triglyceride, that in an α-position, will be labeled, while with the first set of reactions (a), all three hydroxy groups of the glycerol will be occupied by the labeled fatty acid.

In order to determine the distribution of labeled fatty acids in the triglycerides, the latter were subjected to degradation by pancreatic lipase. This lipase is known[6,7] to split triglycerides mainly in the α-position, forming 1,2-diglycerides and 2-monoglycerides. In the case of α-labeled triglycerides this degradation would yield diglycerides with decreased specific activity and monoglycerides, practically devoid of activity. With triglycerides labeled equally in all three positions, on the other hand, no decrease in specific activity will take place.

The method was first tested, using triglycerides labeled by exchange with [1-^{14}C]palmitic acid in the presence of pancreatic lipase, which labels predominantly the α-position (see ref. 5).

The triglyceride was isolated from the reaction mixture by extraction with alcohol–ether (3:1, v/v). The extract, after evaporation, was taken up in acetone and passed through a MgO column to remove free fatty acids. The glycerides in the acetone eluate were then fractionated on a silicic acid column[8] and the triglyceride fraction obtained. Lipolytic degradation was now carried out on the triglyceride by a method similar to that of MATTSON[6]. The labeled triglycerides were dissolved in a small amount of ether and added to a hot solution of 5 % gum accacia in 1 *N* NH_4OH–NH_4Cl buffer, pH 8.0, and dispersed by homogenization in a small blendor.

TABLE I

DEGRADATION OF NEWLY SYNTHESIZED RADIOACTIVE TRIGLYCERIDES DERIVED FROM ADIPOSE TISSUE

Expt.	*Specific activity (counts/min/μequiv. ester)*		
	Before lipolysis	*After lipolysis and subsequent MgO chromatography*	
		20 min lipolysis approx. 40 %	*30 min lipolysis approx. 60 %*
1	322	333	—
2	1455	1405	—
3	460	406	347
4	1625	1635	1460

Degradation of pancreatin-catalyzed exchange-labeled triglycerides

Expt.	*Specific activity (counts/min/μequiv. ester)*		
	Before lipolysis	*After lipolysis and subsequent MgO chromatography*	
		20 min lipolysis approx. 40 %	*30 min. lipolysis approx. 60 %*
1	100	27.5	—
2	250	42	—

2 ml of the resulting emulsion were then incubated with 1.8 ml of the ammonium buffer, 0.2 ml 60 % $CaCl_2$ and 100 mg pancreatin (Nutritional Biochemicals Corp., Cleveland) dispersed in 2 ml water at 37° in a water bath with continuous shaking. Incubation was continued until the lipolysis was about 40–60 % complete (followed by determination of the ester bonds, according to STERN AND SHAPIRO[9]), to obtain a fair proportion of monoglycerides. The glycerides were then extracted and isolated as described above, except that no additional separation of glycerides on silicic acid was undertaken. For the actual test, triglycerides were synthesized by mesenteria of rats previously starved for several days and refed on a high-carbohydrate diet, two days prior to the experiment. The tissues were incubated with [1-^{14}C]palmitic acid in a medium of 5 % albumin in Ca^{++}-free Krebs–Ringer phosphate solution. After 2-h incubation the triglycerides were isolated as described before and subjected to similar lipolytic degradation.

The results summarized in Table I show that while the glycerides obtained from triglycerides labeled by lipase-catalyzed exchange lost the major part of their activity upon subsequent lipolysis, the newly synthesized adipose tissue triglyceride underwent practically no reduction in specific activity by this procedure.

These results indicate that the triglyceride synthesis in adipose tissue follows mainly pathway (a), *i.e.* total esterification of α-glycerophosphate with three molecules of fatty acid.

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Enzymic synthesis of glutaryl-coenzyme A

The activation of succinate to succinyl-CoA[1,2] and of malonate to malonyl-CoA[3–5] by an ATP- and CoA-dependent reaction is well documented. We have found that glutaryl-CoA is synthesized in animal tissues from glutarate in analogous manner according to reaction (1).

$$\text{Glutarate} + \text{ATP} + \text{CoA} \rightleftharpoons \text{glutaryl-CoA} + \text{ADP} + P_i \qquad (1)$$

Abbreviations: CoA, coenzyme A; ATP, adenosine triphosphate; GTP, guanosine triphosphate; ITP, inosine triphosphate; Tris, tris(hydroxymethyl)aminomethane; GSH, glutathione.