A full report of the data and the complete calculations will be published in due time. This work has been supported by *Statens naturvetenskapliga forskningsråd (Swedish natural science research council)*. We wish to thank Dr. Georg Biedermann for helpful advice in the experiments.

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Fatty Acid Esterification in Man during Fat Absorption

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Previous studies in man have demonstrated that dietary cholesterol is largely absorbed through the lymphatics and that the major portion is esterified during the absorption phase.[1-3]

During the investigation of the effect of the dietary fatty acids on the fatty acid composition of the lipids in the thoracic duct lymph in man, it was noted by Blomstrand and Dahlbäck that the lymph triglycerides and the cholesterol esters were highly influenced by the dietary fat.[1] In animal studies good evidence has been reported to support the theory that endogenous and exogenous cholesterol passes into the intestinal mucosa as free choles-

terol and there becomes esterified with certain long chain fatty acids.[4-6]

Experiments in rats have shown that the chylomicron cholesterol ester formation has a specificity for oleic acid relative to the other fatty acids tested.[7] On the other hand, chylomicron triglyceride formation showed no specificity for one fatty acid relative to another with the exception of slight discrimination against stearic acid.

This paper gives a report of investigations conducted in man in order to study the fatty acid specificity of the mechanisms involved in the lymph cholesterol esters and triglyceride formation during fat absorption. The distribution of mass and radioactivity in the fatty acids of cholesterol esters and triglycerides of human thoracic duct lymph is given after feeding a mixture of ^{14}C-labelled palmitic, oleic and linoleic acid as free acids. The results indicate strongly that in man there is a preferential incorporation of oleic acid into lymph cholesterol esters relative to other fatty acids tested.

Experimental. The patient in this study was a forty-eight year old woman with a previous operated breast cancer and with pulmonary metastases. She had no signs of gastrointestinal dysfunction. She was in good condition during this study. In connection with scalene lymph node biopsy the thoracic duct was cannulated with a polyethylene tubing. After fasting overnight the patient was fed a liquid formula meal containing 15 μC palmitic acid-1-^{14}C + 2 g palmitic acid, 15 μC oleic acid-1-^{14}C + 2 g oleic acid and 15 μC linoleic acid-1-^{14}C + 2 g linoleic acid together with 18 g of egg withe and 20 g of dextrose mixed in 150 ml of water. (The labelled material was obtained from Radiochemical Centre, Amersham, England.)

The lymph was collected for one hour periods in plastic containers. The lipids were extracted with chloroform: methanol 2:1 and were separated into their various lipid classes by a combination of column and thinlayer silicic acid chromatography.[8]

A preparative Pye argon chromatograph equipped with a strontium detector and a pyrex glass splitter was used. The glass splitter was connected by means of silicon rubber fittings to a stainless tube filled in the first section with copper oxide and in the second section with reduced iron. The stainless tube was heated to 800°C by means of an electronically controlled furnace. The radioactivity of the $^{14}CO_2$ peak was assayed by a 10 ml internal flow proportional counter[9] at room tem-

perature with argon as the carrier gas and CO_2 added externally to give a final concentration of 5 %. The column used was 1200 mm, 10 mm internal diameter and consisted of 12 % polyethylene glycol succinate, supported on 100—120 mesh acid — and alkaliwashed and silane treated Celite, at a temperature of 160°C.

The output of both the ionization and the proportional counter was fed into a two-channel potentiometric recorder (Texas Model PWS). The areas were measured by cutting out the peaks from the recorder chart and weighing the paper. The radioactivity of the different lipid fractions was measured in an Ekco scintillation counter using a toluene-2,5-diphenyloxazole mixture. After methylation with diazomethane a known amount of labelled material was introduced into the gas chromatograph as described by Blomstrand and Gürtler.[10]

Photographs of the original recorder tracings of dietary fatty acids, the cholesterol ester fatty acids and the triglyceride fatty acids of the lymph are shown in Figs. 1, 2, and 3. The fatty acid composition as well as the distribution of the radioactivity is given in these figures. In Table 1 the distribution of mass and radioactivity in each lipid class is given. The distribution of the radioactivity
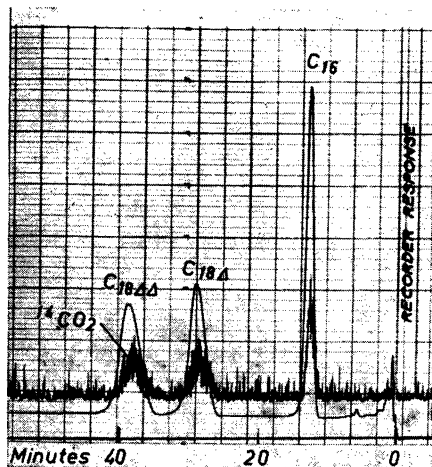

Fig. 1. Analyses of mass (lower curve) and radioactivity $^{14}CO_2$ (upper curve) of dietary fatty acids. Photograph of the original recorder tracing. Polyethylene glycol succinate stationary phase at 160°C. An ionization chamber was used for determination of the mass, and a flow proportional counter for determination of $^{14}CO_2$.
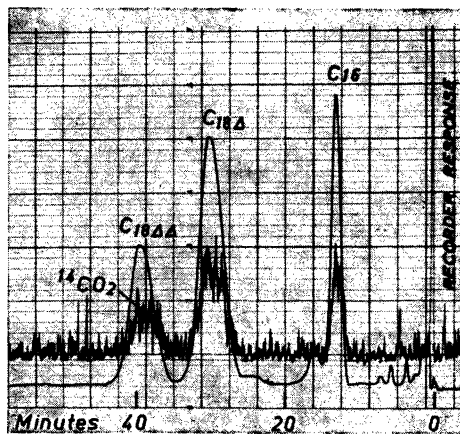

Fig. 2. Analyses of mass (lower curve) and radioactivity $^{14}CO_2$ (upper curve) of human lymph cholesterol esters after feeding the diet shown in Fig. 1. Conditions same as in Fig. 1.
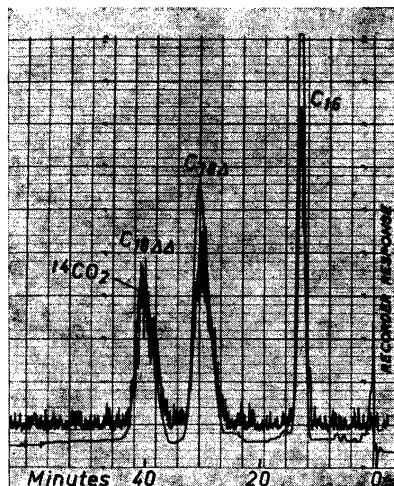

Fig. 3. Analyses of mass (lower curve) and radioactivity $^{14}CO_2$ (upper curve) of human lymph triglycerides after feeding the diet shown in Fig. 1. Conditions same as in Fig. 1.

in the cholesterol esters differed from that in the test meal and from that of the lymph triglycerides. The fatty acids of the cholesterol esters analyzed showed that relatively more radioactivity was found in oleic acid than in any of the other acids being studied.

The specific radioactivity of each of the fatty acids of the cholesterol esters and tri-

Table 1. Distribution of mass and radioactivity and specific activity among the fatty acids of the diet and the chlesterol esters and triglycerides of the thoracic duct lymph of a patient after feeding equal amounts of palmitic acid-1-^{14}C, oleic acid-1-^{14}C and linoleic acid-1-^{14}C as free acids. Values are expressed as percentage of total fatty acid methyl esters and percentage of total radioactivity.

	Mass distribution			Radioactivity distribution			Specific activities (counts/sec/mg)		
	16:0	18:1	18:2	16:0	18:1	18:2	16:0	18:1	18:2
Dietary fatty acids	34.6	30.1	35.3	34.6	31.5	33.9	183.9	183.6	183.7
Cholesterol esters	20.0	43.5	28.4	22.3	50.3	27.4	75.2	78.0	65.0
Triglycerides	29.5	38.8	29.4	29.7	37.5	32.8	155.1	149.4	171.8

glycerides compared with corresponding specific radioactivity in the fed fat is shown in Table 1. The results show that about 60 % of each of the fatty acids in the cholesterol esters is of endogenous origin but in the case of the triglycerides only about 15 % is of endogenous origin.

Comments. The results of the present investigation demonstrate conclusively that 4 h after the ingestion of a mixture of labelled palmitic, oleic and linoleic acid more than 50 % of the total radioactivity in the lymph cholesterol esters is represented by oleic acid. The specific activity of oleic acid in the lymph cholesterol esters is also higher than those of palmitic and linoleic acid. To assure ourselves that in the case of palmitic and linoleic acid we were not dealing with a slower incorporation we have followed the radioactive fatty acid distribution pattern of both cholesterol esters and triglycerides every second hour during the whole absorption process. We have not been able to show any significant changes in this distribution pattern in this patient. A possible explanation of the results would be an interconversion of palmitic or linoleic acid into oleic acid during the absorption process. In other lymph patients fed single labelled fatty acids, no such interconversion has been noted.[11] Furthermore in a recent investigation the results obtained were confirmed after feeding a mixture of ^{14}C-labelled palmitic, stearic, oleic, and linoleic acids to another patient.

We conclude, therefore, that in man chylomicron cholesterol ester formation shows marked specificity for oleic acid relative to other fatty acids tested. Similar results have been recently reported in experiments with rats.[7] In the formation of lymph triglycerides no such specificity for one fatty acid relative to another was noted. The metabolic significance of this fact is difficult to assess. It is possible that the oleic acid specificity for lymph cholesterol ester formation affects the metabolism of the liver cholesterol esters.

In this connection it is well worth to mention that in chicken cholesterol feeding or stilboestrol treatment provokes an increased oleic acid concentration in the cholesterol esters of plasma and aorta.[12]

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